



July 2017

## Aflatoxins B/G and Ochratoxin A in Puffed Millet ~ manual and automated ~

Do you have a special matrix that we should test for mycotoxins? Please let us know and write an e-mail to: [mycotoxins@LCTech.de](mailto:mycotoxins@LCTech.de)

### Sample Preparation

MYCOTOXINS

#### Millet

Millet belongs to the family of sweet grass (*Poaceae*) and is mainly cultivated in tropical and subtropical areas. There are over 600 million types of millet worldwide. Thereby, depending on the type, the color varies between white-gray, yellow and red-brown. The tiny, small-sized millet seeds provide a very high nutrient density, are completely gluten-free, and a wonderful ingredient for a fast cooking. In many parts of Africa and Asia, millet is one of the most important basic foodstuffs.

Meanwhile, millet is also cultivated in many European countries, including Germany. When importing, however, our quality requirements are not always fulfilled. This is shown by the border controls of the EU where aflatoxins and ochratoxin A repeatedly lead to rejection.

*One for all – fast and efficient*

#### Combined Immunoaffinity Column Afla-OtaCLEAN for Clean-up of Mycotoxins

Aflatoxins and Ochratoxin A are naturally occurring mycotoxins and are produced by fungi in wet or rather wrong storage. They are often found together in many foods and feeds. In order to reduce the workload and to halve the working time, a way of testing several mycotoxins in one single operation was needed. For this reason, LCTech developed the combined immunoaffinity column Afla-OtaCLEAN for the clean-up of aflatoxin B1, B2, G1, G2 and ochratoxin A. Since LCTech produces both antibodies and clean-up columns, comprehensive quality testing throughout the entire manufacturing process ensures high product quality.

All clean-up columns are suitable for automated processing and are dedicated for use in the LCTech devices FREESTYLE SPE or FREESTYLE ThermELUTE™. Time-consuming and tedious tasks are not a problem for the FREESTYLE system. These tasks are processed reliably and consistently during day, night and over the weekend. Beside the LCTech immunoaffinity and SPE columns the FREESTYLE SPE can be used for all types of mycotoxin columns of leading manufacturers.



Immunoaffinity columns Afla-OtaCLEAN

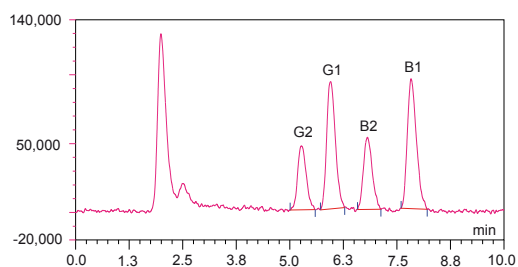
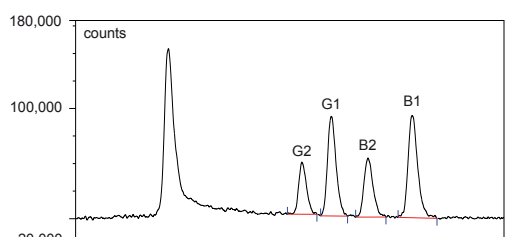
## Protocol of Manual Processing

Homogenise 10 g of puffed millet and add 2 g sodium chloride, 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane to remove fat and oil. The extraction should be performed for 10 - 15 minutes.

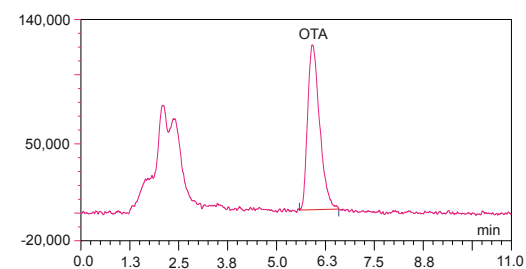
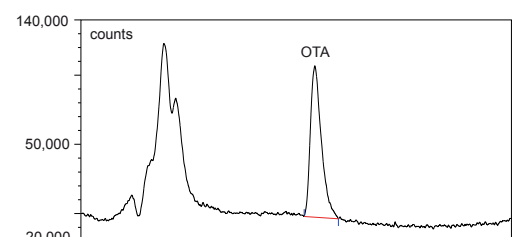
Filtrate the raw extract and dilute 2 mL with 12 mL PBS (contains 8 % Tween). Load the sample completely onto an immunoaffinity column Afla-OtaCLEAN. Afterwards wash the column with 2 x 5 mL deionised water and load this solution onto the IAC-column, too.

Dry the column and elute the toxin with 2 mL methanol. Keep in mind, that the methanol incubates for 5 minutes into the column bed, in order to dissolve the antibody toxin bond completely. Dilute the sample to HPLC conditions and measure it afterwards.

## Chromatograms



Black: puffed Millet 10 ppb  
Red: Aflatoxin Standard 10 ppb (2 ng / 2 mL)



Black: puffed Millet 10 ppb  
Red: Ochratoxin A Standard 10 ppb (2 ng / 2 mL)

## HPLC-Conditions

(Aflatoxins B/G / Ochratoxin A)

Mycotoxin:	Aflatoxin B/G	Ochratoxin A
HPLC:	isokratisch	isocratic
Column Oven:	36 °C	40 °C
Separation Column:	RP C-18 (P/N 10544)	RP EC 125/3 nucleosil 120-3 C18
Flow Rate:	1,2 mL/min	0,6 mL/min
Eluent:	HPLC-water/methanol/acetonitrile (60/30/15 (v/v/v))	HPLC-water/methanol/acetonitrile (40/55/5 (v/v/v)) + 1 % acetic acid
Fluorescence Detection:	Derivatisation with UVE Photochemical Reactor	without Derivatisation
Excitation Wavelength:	365 nm	335 nm
Emission Wavelength:	460 nm	465 nm

## Recovery Rates

Content of Aflatoxins B/G / Ochratoxin A in Puffed Millet

Aflatoxins B/G / Ochratoxin A	B1	B2	G1	G2	OTA
Standard*	100	100	100	100	100
Recovery Rate** Puffed Millet, 10 ppb	90	95	94	86	88

\*Standard is set = 100 %, \*\*Corrected with non-spiked sample/  
The results correspond to the performance specifications of EC 401/2006 (Section 4.3.1)

## These LC Tech products were used:

Afla-OtaCLEAN, Immunoaffinity Column  
for Aflatoxins B/G and Ochratoxin A  
P/N 11022 / 11771

HPLC Separation Column RP C-18  
P/N 10544

UVE, Photochemical Reactor  
P/N 10519

FREESTYLE SPE, Robotic System  
for Automated Sample Preparation  
P/N 12663 / 12668

