



September 2017

## Aflatoxins B/G and Ochratoxin A in Chickpeas ~ 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

#### Chickpeas

Chickpea is not just a funny word: The small „nutrient bombs“ have been consumed for around 8000 years, because they contain lots of proteins, minerals and trace elements. Besides, they are rich in B vitamins and fiber: a perfect choice for all vegans, health and and figure-conscious consumers. Chickpeas belong to the family of pulses and have a slightly nutty taste. The chickpea can also be cultivated in Europe, but they grow better in a dry and warmer climate. Therefore, they are mainly imported from other countries.

When importing, however, border controls of the EU show that the European limits for mycotoxins in food and feed are not always met. It is not uncommon that exceedances of aflatoxin B/G and ochratoxin A are followed by rejections of the commodities.

#### One for Both: Aflatoxins B/G and Ochratoxin A Immunoaffinity Columns for the Clean-up of Mycotoxins



Aflatoxins B/G and ochratoxin A are often found combined in food and feed. The immunoaffinity columns Afla-OtaCLEAN from LCTech allow the simultaneous clean-up of both mycotoxins. The working mode is based on the principles of immunoaffinity. The material within the columns is covered with antibodies which are directed against aflatoxins B/G and against ochratoxin A. Upon application of a crude extract onto the column, the toxins are retained, whereas the remaining matrix components pass through the column. After a washing step, the aflatoxins and ochratoxin A can be eluted quantitatively from the column with methanol and subsequently measured with HPLC.

The Afla-OtaCLEAN columns can be operated manually or automated, for example with the LCTech robotic system FREESTYLE SPE. Besides the immunoaffinity columns LCTech also offers columns for other SPE applications, such as the DONeX columns for the analysis of deoxynivalenol, the BioteX columns for the analysis of biotin/vitamin B7 or the Elufix florisil ready-to-use glass columns (filled and unfilled) for the H53-method.

## Protocol of Manual Processing

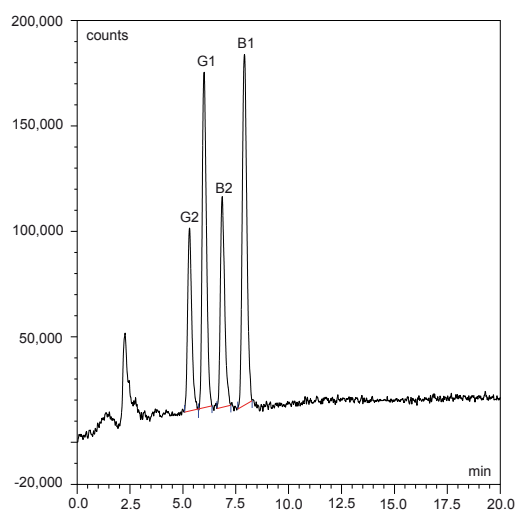
Homogenise 10 g of chickpeas and add 1 g of sodium chloride. Extract the sample through 50 mL methanol/water (80/20 (v/v)) and 25 mL n-hexane in order to remove fat and oils. The extraction should be conducted for 10 minutes.

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

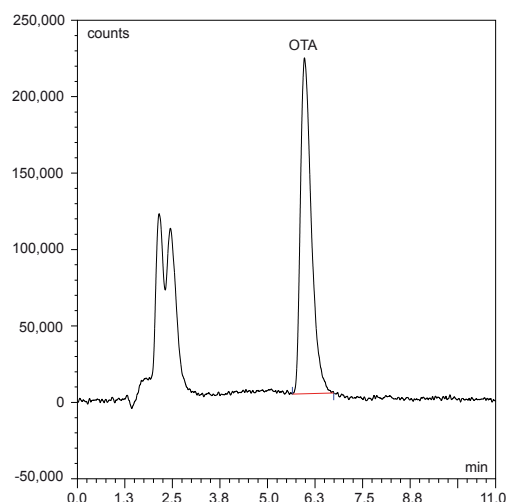
Dry the column by flushing air through it and elute the toxins afterwards with 2 mL methanol. Keep in mind that the column bed is incubated with methanol for at least 5 minutes in order to ensure the complete denaturation of the antibody.

Dilute the sample to eluent conditions and measure it afterwards via HPLC with fluorescence detection or LC-MS.

## Chromatograms



Aflatoxins B/G Chickpeas 10 ppb



Ochratoxin A Chickpeas 10 ppb

## 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 Chickpeas

Aflatoxins B/G / Ochratoxin A	B1	B2	G1	G2	OTA
Standard*	100	100	100	100	100
Recovery Rate** Chickpeas, 10 ppb	92	95	90	86	93

\*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