



October 2017

## Aflatoxins B/G, Ochratoxin A and Zearalenone (A-O-Z) in Corn ~ separate and combined ~

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

#### Clean-up 3 in 1

Considering that mycotoxins are often found together in samples, the clean-up of several toxins in one workflow leads to an enormous time and solvent saving. Furthermore, if the clean-up is done with a small 3 cm column format, the saving is enhanced considerably.

LCTech developed a SMART column format which can be individually combined dependent on the respective toxins. Due to that fact you can for example combine the immunoaffinity columns AflaCLEAN SMART, OtaCLEAN SMART and ZeaCLEAN SMART either for the clean-up of one, two or even three toxin groups at once.

The SMART columns are AOAC-conform. Since LCTech produces both, antibodies and clean-up columns, comprehensive quality testing throughout the entire manufacturing process ensures high product quality. The clean-up columns are, suitable for the automated processing in the FREESTYLE ThermELUTE™, if used separately.

#### SMART combined



✓ Aflatoxins B/G

✓ Ochratoxins A

✓ Zearalenone

*Just stack the three columns and they will be instantly ready for the manual clean-up of aflatoxins B1, B2, G1, G2, ochratoxin A and zearalenone.*



## Protocol of Manual Processing

Homogenise 20 g of corn and extract the sample with 100 mL methanol/water (80/20 (v/v)). Depending on the extraction apparatus, carry out the extraction for 10 - 20 minutes using a magnetic stirrer or for 3 - 5 minutes using an Ultraturrax to avoid lower extraction efficiencies. Filtrate the raw extract and dilute 5 mL of it with 35 mL PBS. Afterwards filtrate the diluted extract again with a Whatman glass fiber filter in order to remove turbidites.

Stack the SMART columns on the top of the other. Please consider to position the ZeaCLEAN SMART column always at the bottom, so that the AflaCLEAN SMART column can bind the aflatoxins and the OtaCLEAN the ochratoxins beforehand. Load 10 mL of the sample (represents 0,25 matrix) with a flow rate of 1.5 mL onto the corresponding SMART column. Wash the sample reservoir with 2 mL deionised water and load this solution also onto the column.

Elute the columns either with acetonitrile, or alternative the AflaCLEAN SMART and OtaCLEAN SMART with methanol and only the ZeaCLEAN SMART with acetonitrile. Keep in mind, that the elution solution incubates for 5 minutes into the column bed, before collecting the eluate. Dry the column with an air flush and collect the rest of the eluate, too.

HPLC-Conditions (Aflatoxins B/G / Ochratoxin A / Zearalenone)			
Mycotoxin:	Aflatoxin B/G	Ochratoxin A	Zearalenone
HPLC:	isocratic	isocratic	gradient
Column Oven:	36 °C	40 °C	38 °C
Separation Column:	RP C-18 (P/N 10544)	RP EC 125/3 nucleosil 120-3 C18	RP C-18 (P/N 10544)
Flow Rate:	1,2 mL/min	0,6 mL/min	00.00 - 16:90: 1.0 mL/min (75% A; 25% B) 17.00 - 19:90: 1.0 mL/min (100 % A; 0% B) 20.00: - 26:00: 1.0 mL/min (75% A; 25% B)
Eluent:	HPLC-water/methanol/ acetonitrile (60/30/15 (v/v/v))	HPLC-water/methanol/ acetonitrile (40/55/5 (v/v/v)) + 1 % acetic acid	<b>Eluent A:</b> HPLC-water/ methanol (45/55 (v/v)) + 2 % acetic acid <b>Eluent B:</b> water/acetonitrile (95/5 (v/v))
Fluorescence Detection:	Derivatisation with UVE Photochemical Reactor	without Derivatisation	without Derivatisation
Excitation Wavelength:	365 nm	335 nm	274 nm
Emission Wavelength:	460 nm	465 nm	440 nm

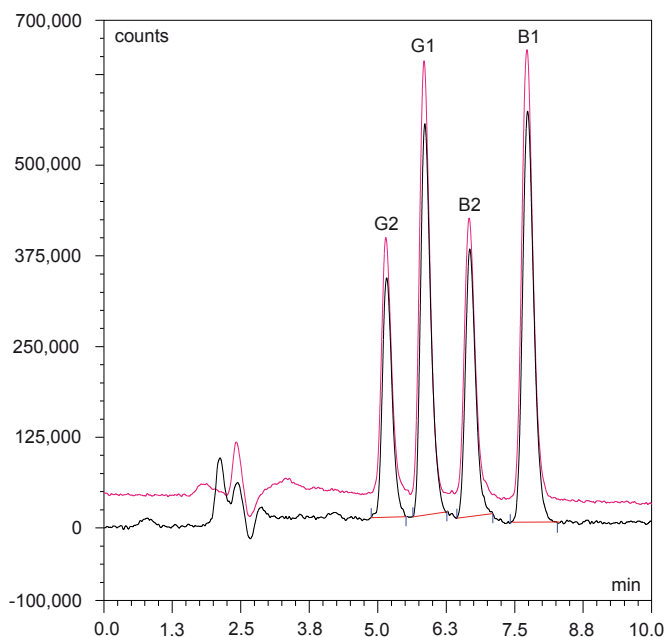
Recovery Rates Content of Aflatoxins B/G, Ochratoxin A and Zearalenone in Corn						
Aflatoxins B/G / Ochratoxin A Zearalenone	B1	B2	G1	G2	OTA	ZEA (250 ppb)
Standard*	100	100	100	100	100	100
Recovery Rate** Corn, 10 ppb	84	91	84	84	92	93

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

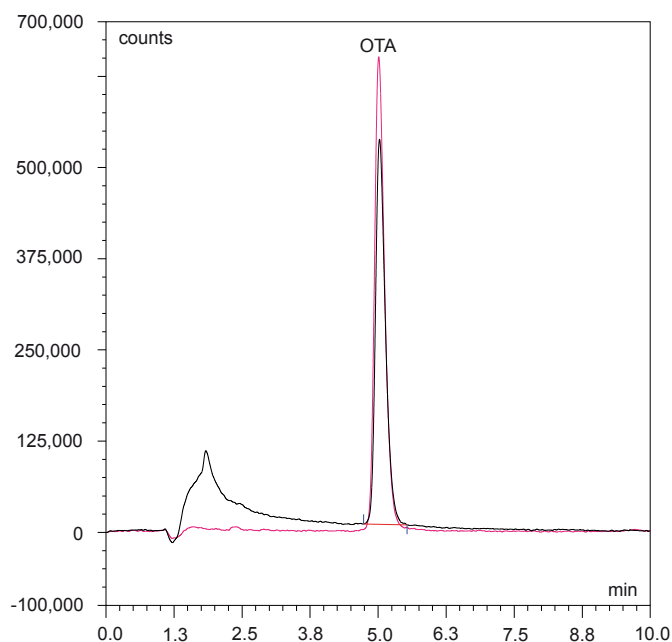


Find chromatograms  
on the next page.

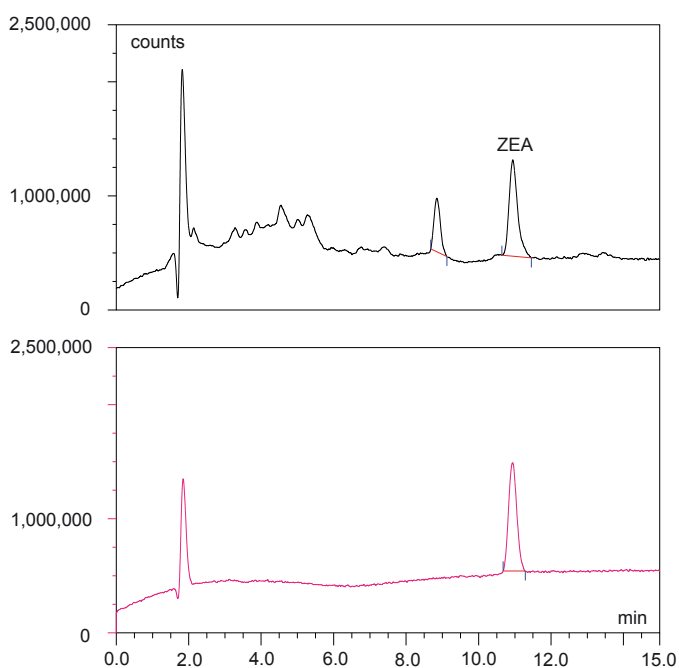
## Chromatograms



Black: Corn sample 10 ppb total toxin spiked before  
Red: Standard 10 ppb total toxin (4 ppb (B1/G1), 1 ppb (B2/G2))



Black: Corn sample 10 ppb OTA spiked before  
Red: Standard 10 ppb



Black: Corn sample 200 ppb ZEA spiked before  
Red: Standard 200 ppb



SMART immunoaffinity columns  
with column tip

### Flexible - Combinable - Efficient - Fast - SMART

Whether A-Z, O-Z, A-O or A-O-Z, each combination is possible. Due to the fact that the processes are summarised, the total processing time and the workload are reduced significantly. Therefore, the eluate for subsequent analysis is ready in less than 15 minutes (exclusive extraction time).

### These LCTech products were used:

AflaCLEAN, Immunoaffinity Column for Aflatoxins B/G  
P/N 12862 / 12863

OtaCLEAN, Immunoaffinity Column for Ochratoxin A  
P/N 13346 / 13351

ZeaCLEAN, Immunoaffinity Column for Zearalenone  
P/N 14741 / 14742

HPLC Separation Column RP C-18  
P/N 10522

UVE, Photochemical Reactor  
P/N 10519