

# Matrix of the Month

February, 2014:  
**Aflatoxins B/G and  
Ochratoxin A  
in Drinking Cocoa**



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

## Protocol

20 g homogenised drinking cocoa are mixed with 2 g sodium chloride and extracted with 100 mL 80/20 (methanol/water (v/v)) and 50 mL n-hexane (20-30 min).

In case of low extraction efficiency, the matrix could be changed to sample amount (10 g/100 mL). The extract is filtrated and centrifuged for 10 min at 2000xg to support phase separation of aqueous and n-hexane phase. 2 mL of the aqueous phase (lower phase) are diluted with 12 mL PBS buffer containing 8% Tween20.

The extract is applied to the immunoaffinity column Afla-OtaCLEAN, afterwards the sample reservoir is rinsed with 10 mL of deionised water. This washing solution is also applied to the immunoaffinity column.

The column is dried and eluted with methanol (2 mL). The methanol should incubate on the column bed for 5 minutes to ensure that the antibody is entirely denaturated.

The eluate is diluted to HPLC conditions and measured.

## HPLC Conditions

### Aflatoxins B/G

HPLC: Dionex Ultimate 3000 isocratic

Column oven: 36 °C

Separation column: RP C18

Flow rate: 1.2 mL/min, water/methanol/acetonitrile (60/30/15 (v/v/v))

Fluorescence detection: photochemical derivatisation with UVE

Excitation wavelength: 365 nm

Emission wavelength: 460 nm

### Ochratoxin A

HPLC: Dionex Ultimate 3000 isocratic

Column oven: 40 °C

Separation column: EC125/3 Nucleosil 120-3 C-18 RP

Flow rate: 0.6 mL/min, water/methanol/acetonitrile (40/55/5 (v/v/v)) + 1 % acetic acid

Fluorescence detection: -

Excitation wavelength: 335 nm

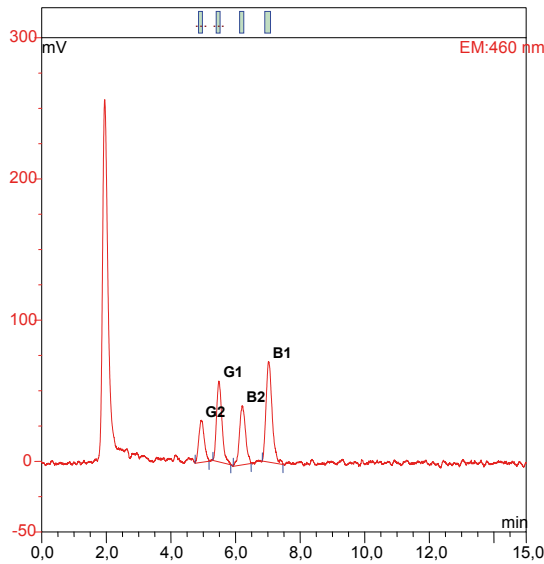
Emission wavelength: 465 nm

Recovery Rates

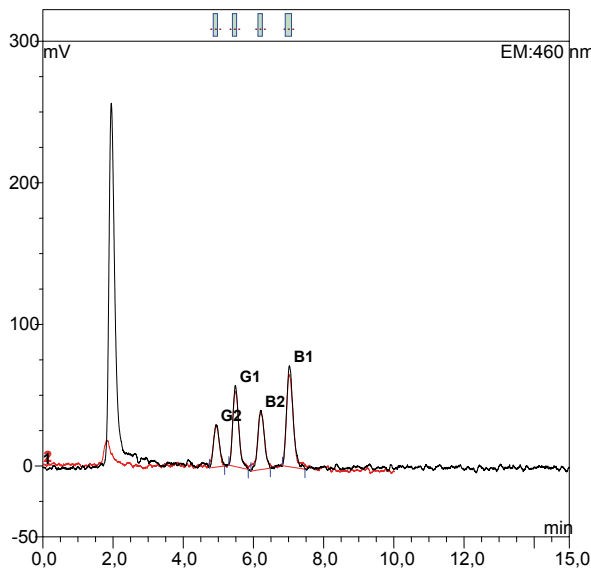
Content of Aflatoxins B1, B2, G1 and G2 as well as Ochratoxin A in Drinking Cocoa					
	Aflatoxin				Ochratoxin A
	B1	B2	G1	G2	
Standard*	100	100	100	100	100
Recovery rate* Drinking Cocoa 10 ppb	99	94	99	90	85

\* Standard is set = 100 % , \*\* corrected with non-spiked sample

Chromatograms



**Aflatoxins:  
Drinking Cocoa, 10 ppb**

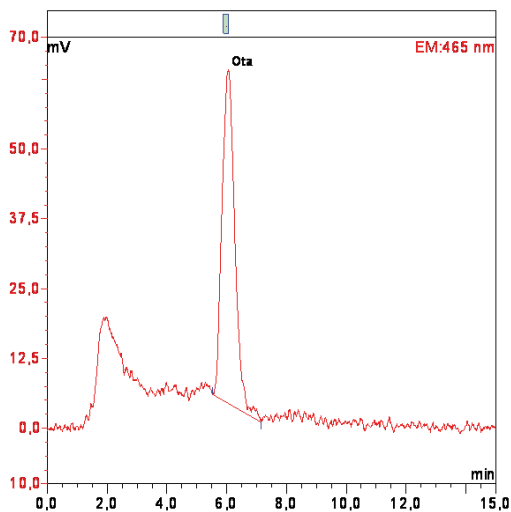


**Aflatoxins:  
Overlay of both chromatograms**

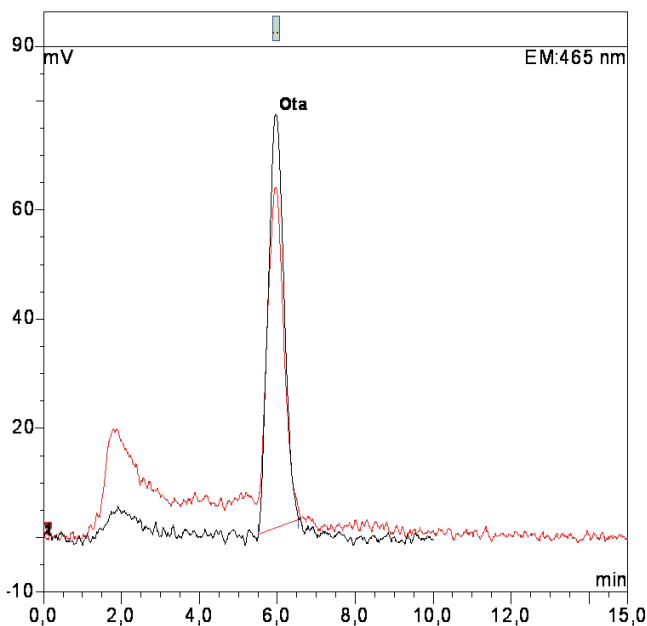
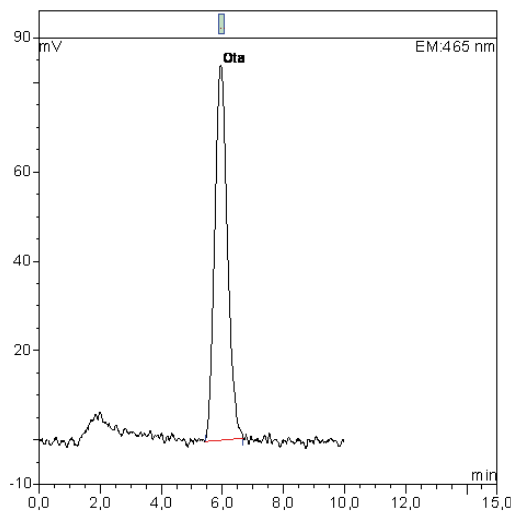
Further chromatograms →

Chromatograms

**Ochratoxin A:  
Drinkin Cocoa, 10 ppb**



**Ochratoxin A:  
Standard, 10 ppb, (4 ng/2 mL)**



**Ochratoxin A:  
Overlay of both chromatograms**

These LCTech products were used:

AflaCLEAN,  
Immunoaffinity column  
for the Aflatoxins B1, B2, G1, G2

P/N 10514

UVE,  
Photochemical reactor  
for the analysis of Aflatoxins

P/N 10519

HPLC column,  
for the analysis of Aflatoxins

P/N 10522

OtaCLEAN,  
Immunoaffinity column  
for Ochratoxin A

P/N 10515

Do you have further questions?  
Please simple write an e-mail to [info@LCTech.de](mailto:info@LCTech.de)