

Matrix of the Month

December 2014:

Aflatoxins B/G and Ochratoxin A in Almond Biscuit



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!

Protocol

20 g homogenised almond biscuits are mixed with 2 g sodium chloride and extracted with 100 mL 80/20 (methanol/water (v/v)) and 50 mL n-hexane, by stirring for 10 minutes. The extract is filtrated and 10,5 mL are diluted with 64,5 mL PBS buffer. In case of turbidity remove them with filtration or centrifugation.

Apply 50 mL to the immunoaffinity column Afla-OtaCLEAN with a maximum flow rate of 2 mL / min. Rinse afterwards the sample reservoir with 10 mL of deionised water. Apply this washing solution onto the IAC column, too. Dry the column and elute with 2 mL methanol. Take care that the methanol incubates within the column bed for 5 minutes to ensure that the antibody is entirely denaturated and the toxin is released.

Dilute the eluate to HPLC conditions and measure by HPLC-FLD or equivalent.

HPLC Conditions

Aflatoxins B, G

HPLC: Isocratic

Column oven: 36 °C

Separation column: RP C18
(e.g. P/N 10522)

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

Fluorescence detection with
post column derivatisation
(photochemical with UVE)

Excitation wavelength: 365 nm

Emission wavelength: 460 nm

Ochratoxin A

HPLC: Isocratic

Column oven: 40 °C

Separation column: RP EC 125/3
nucleosil 120-3 C18

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

Fluorescence detection without
post column derivatisation

Excitation wavelength: 335 nm

Emission wavelength: 465 nm

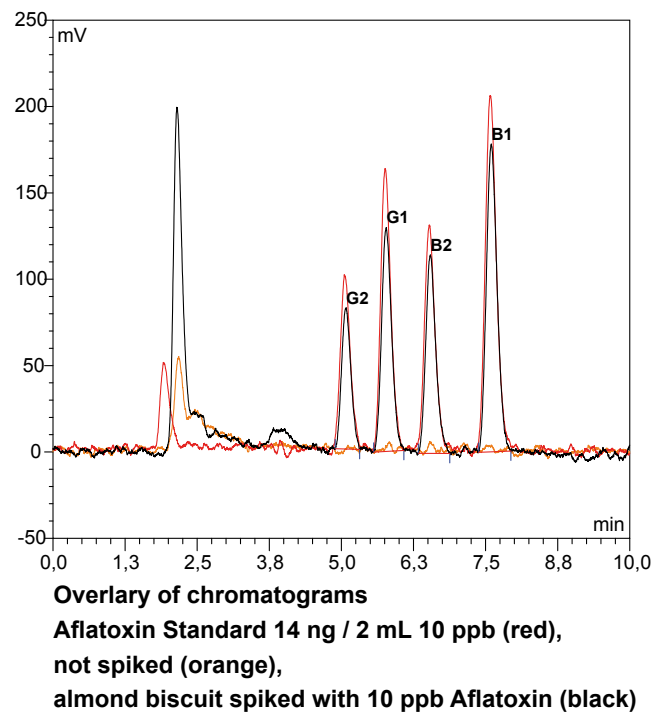
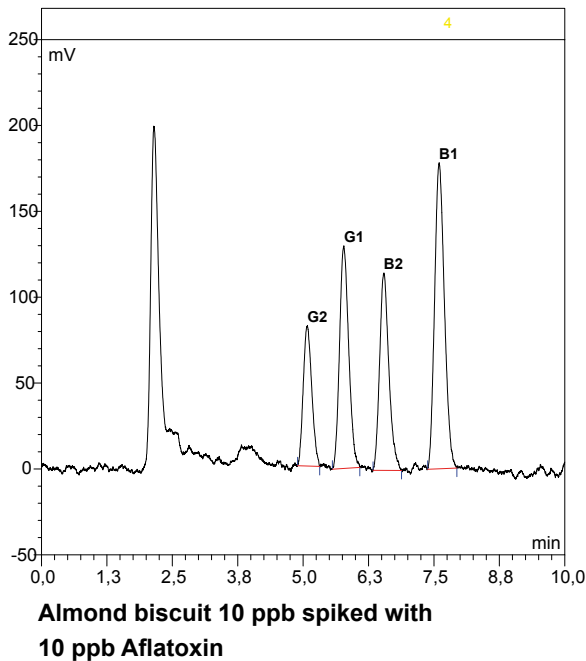
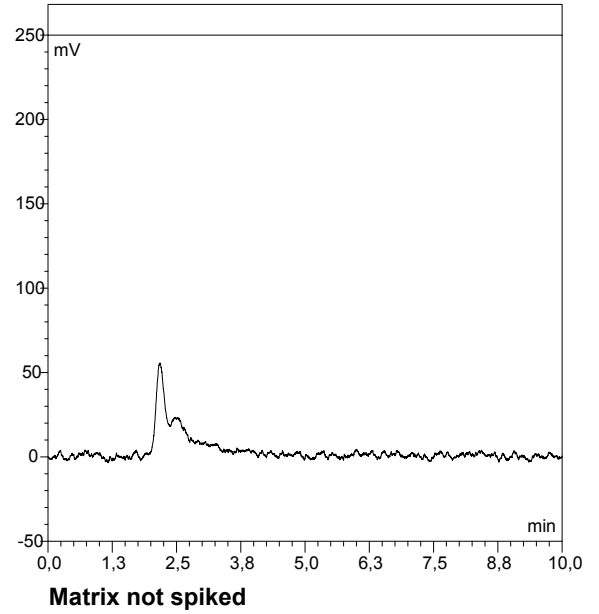
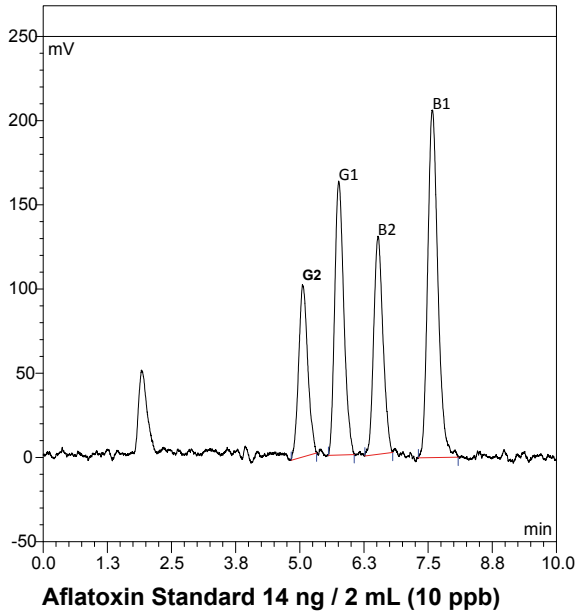
Recovery Rates

Content of Aflatoxins B1, B2, G1, G2 and OTA in Almond Biscuit					
Aflatoxin	B1	B2	G1	G2	OTA
Standard*	100	100	100	100	100
Recovery rate** Almond biscuit 10 ppb (10 ppb = 10 µg / kg Matrix)	90	96	89	89	99

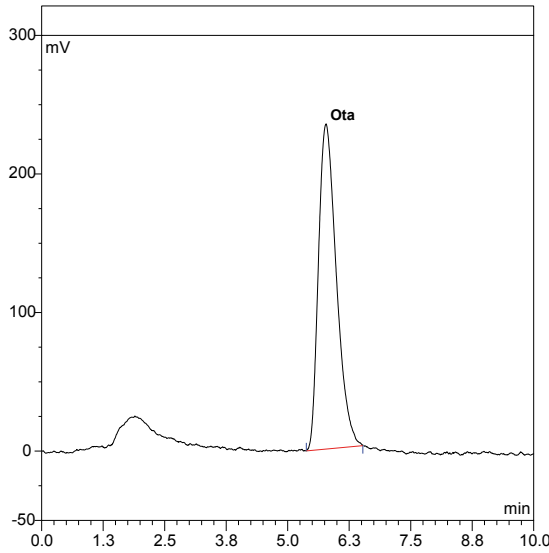
* Standard is set = 100 % , ** corrected with non-spiked sample



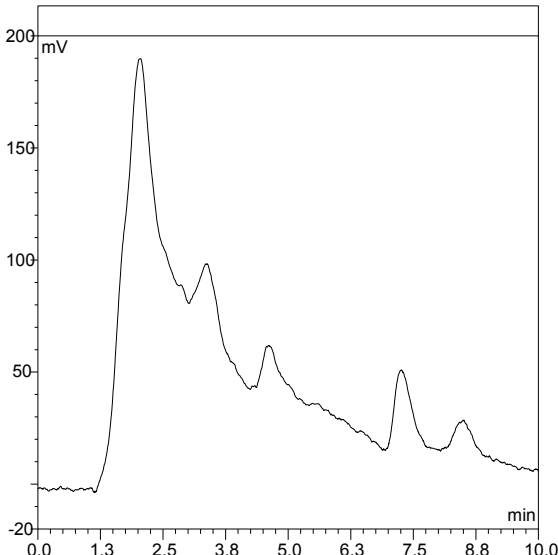
Chromatograms



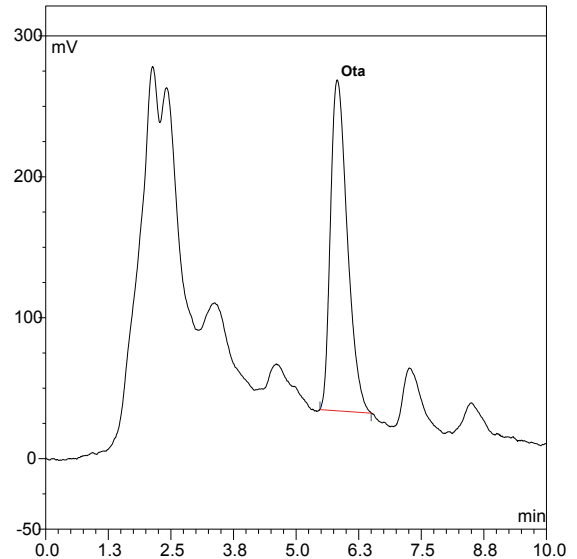
Chromatograms



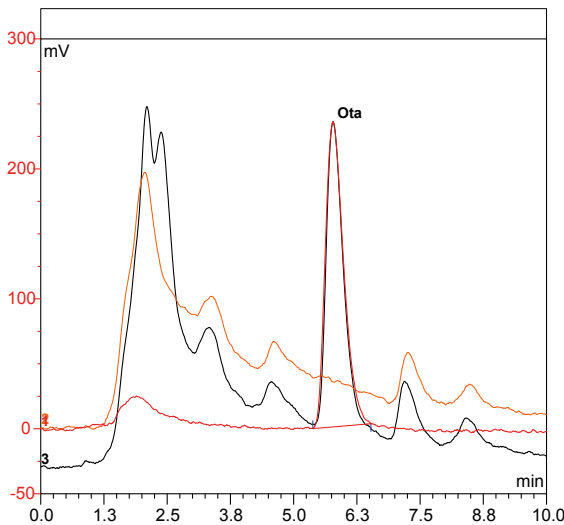
Ochratoxin A, Standard 14 ng / 2 mL (10 ppb)



Matrix not spiked



Almond biscuit, spiked with 10 ppb Ochratoxin A



Overlay of chromatograms
 Standard (red), not spiked (orange)
 Almond biscuit, spiked with 10 ppb
 Ochratoxin A (black)

These LCTech products were used:

Afla-OtaCLEAN,
 Combined column
 for Aflatoxin and Ochratoxin
 P/N 11022 / 11771

UVE,
 Photochemical reactor
 for the analysis of Aflatoxins
 P/N 10519

HPLC column,
 for mycotoxin analysis
 P/N 10522

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Do you have further questions?
 Please just write an e-mail to info@LCTech.de