Mycotoxins: Sample Preparation and Analysis

Matrix of the Month

February 2015:

Aflatoxins B/G and Ochratoxin A in Hemp Seed

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 hemp seed are mixed with 2 g sodium chloride and extracted with 100 mL 80/20 (methanol/water (v/v)) and 50 mL n-hexane.

Filtrate the extract and centrifuge it for 10 minutes at 2000 x g facilitating the phase separation between the aqueous and the n-hexane phase. Dilute 10.5 mL of the aqueous phase (the lower phase) with 64.5 mL PBS buffer and filtrate the diluted extract with a fibre glass filter to remove any turbidities.

Apply 50 mL of the extract (equal to 1.4 g) to the immunoaffinity column Afla-OtaCLEAN and rinse afterwards the sample reservoir with 10 mL of de-ionised 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 and measure the eluate to HPLC conditions.

HPLC Conditions

Aflatoxins B, G	**Ochratoxin A**
HPLC: Isocratic	HPLC: Isocratic
Column oven: 36 °C	Column oven: 40 °C
Separation column: RP C18 (e.g. P/N 10522)	Separation column: RP C18
Flow rate: 1.2 mL/min, water/methanol/ acetonitrile (60/30/15 (v/v/v))	Flow rate: 0.6 mL/min, HPLC-water/methanol/ acetonitrile (40/55/5) + 1% acetic acid
Fluorescence detection with post column derivatisation (photochemical with UVE)	Fluorescence detection without post column derivatisation
Excitation wavelength: 365 nm	Excitation wavelength: 335 nm
Emission wavelength: 460 nm	Emission wavelength: 465 nm

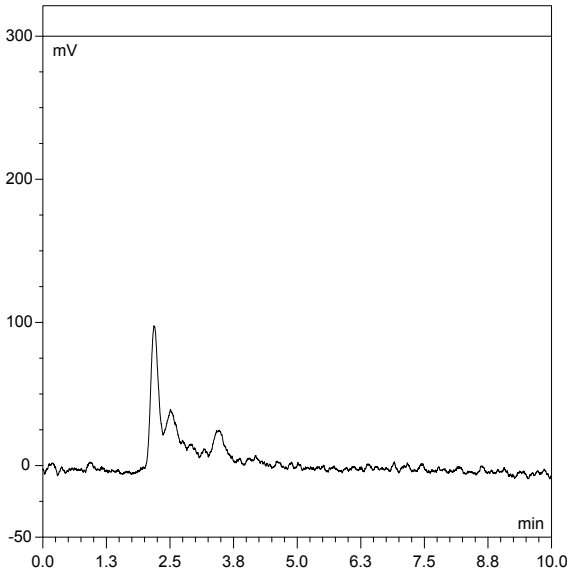
Recovery Rates & Chromatograms →

Recovery Rates

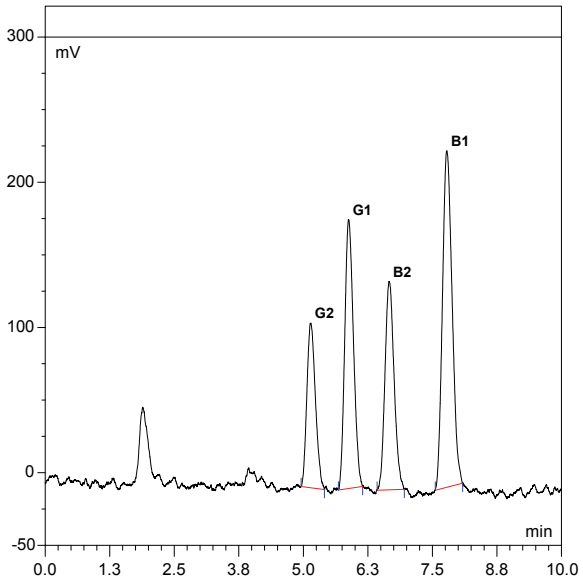
Content of Aflatoxins B1, B2, G1, G2 and OTA in Hemp Seed					
Aflatoxin	**B1**	**B2**	**G1**	**G2**	**OTA**
Standard*	100	100	100	100	100
Recovery rate** Hemp Seed 10 ppb (10 ppb = 10 µg / kg Matrix)	91	95	92	91	91

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

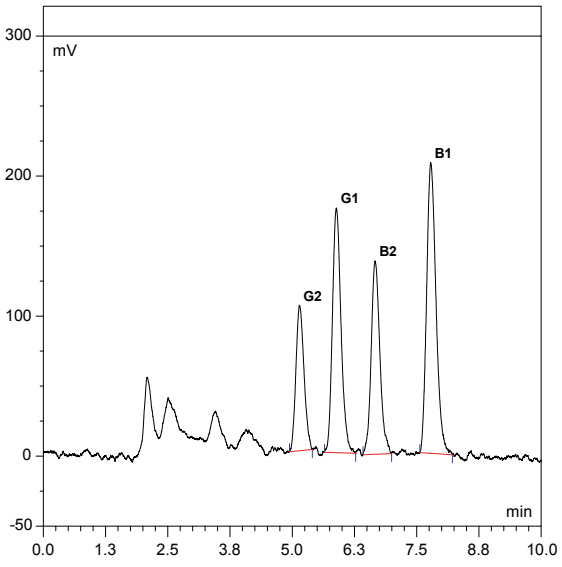
Chromatograms



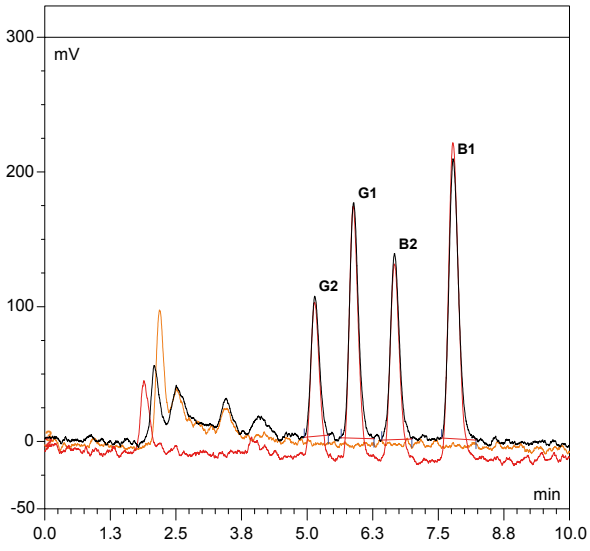
Hemp seed (not spiked)



Aflatoxin standard 14 ng / 2 mL (10 ppb)



Hemp seed spiked with 10 ppb Aflatoxin

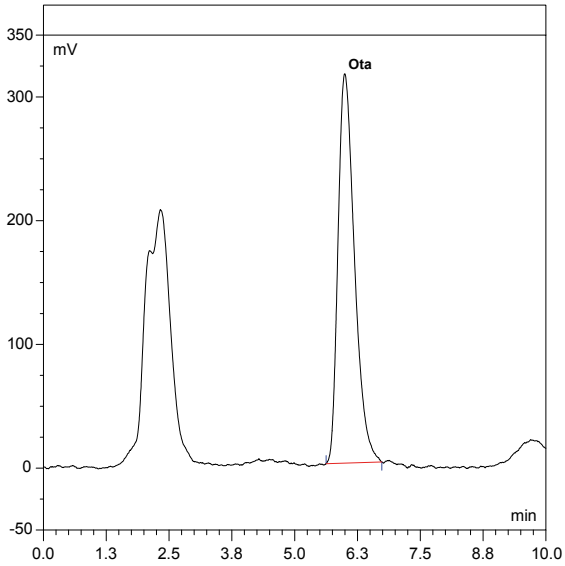


Overlay of chromatograms
Standard (red) (14 ng / 2 mL (equal to 10 ppb)),
Hemp seed 10 ppb (black),
Hemp seed not spiked (orange)

Chromatograms →

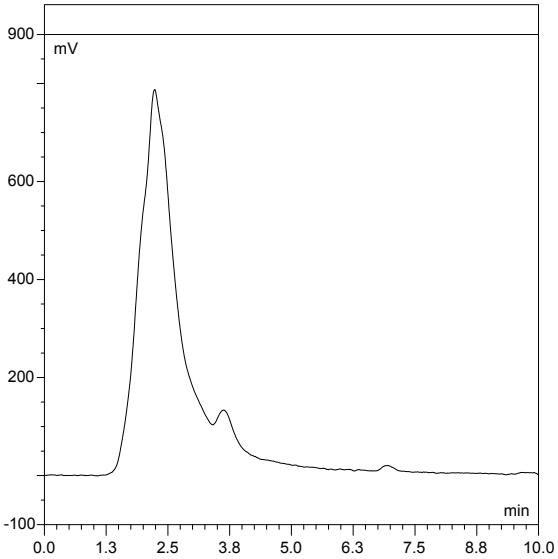
LCTech GmbH Bahnweg 41 D-84405 Dorfen Tel. +49 8081 9368-0 Fax +49 8081 9368-10 www.LCTech.de info@LCTech.de

Chromatograms

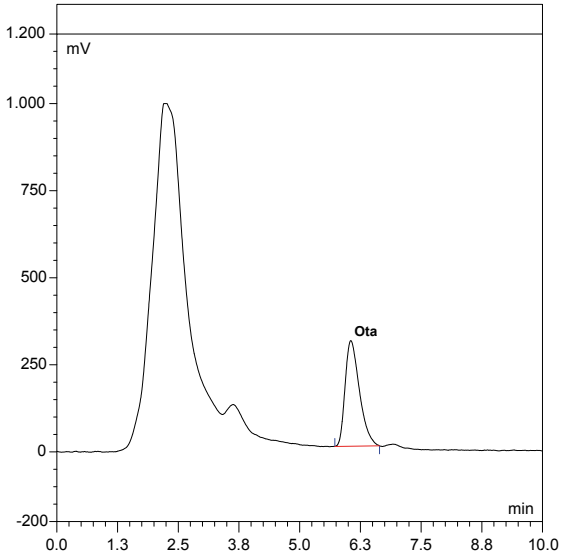


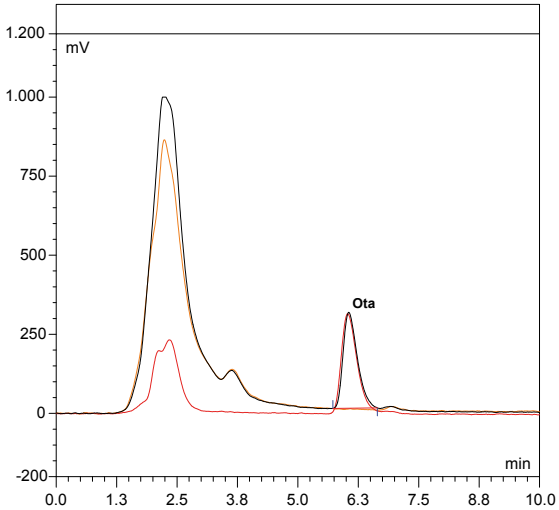
Ochratoxin A, standard 14 ng / 2 mL (10 ppb)

Hemp seed, spiked with 10 ppb Ochratoxin A



Hemp seed, not spiked





Overlay of chromatograms
Standard 14 ng / 2 mL (red), Hemp seed spiked with 10 ppb Ochratoxin A (black), Hemp seed not spiked (orange)

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

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

LCTech GmbH Bahnweg 41 D-84405 Dorfen Tel. +49 8081 9368-0 Fax +49 8081 9368-10 www.LCTech.de info@LCTech.de

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