Mycotoxins: Sample Preparation and Analysis

Matrix of the Month

May 2014:

Ochratoxin A in Beer

OTA-Analysis during the half-time break!

Sample loading, rinsing and eluting in less then 15 minutes!

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 mL beer are degassed by sonication.

8 mL of 3% NaHCO3 solution are added to buffer the mostly low ph-value of beer sample.

Filtrate the buffered beer sample with 0.2 µm injection filter (PVDF) to remove any turbidity.

10 mL filtrated extract are diluted with 40 mL PBS buffer (pH 7.2).

Alternative you can also use smaller sample volumes (e.g. 2 mL + 8 mL buffer).

If there is a precipitate by mixing with buffer, filtrate or centrifugate the sample volume to remove turbidity. After opening the column the storage buffer has to drip off. Take 10 mL of diluted raw extract (depend on measuring sensitivity) and apply them to the OtaCLEAN SMART column. A marginal low or high pressure can be mounted in all steps, when liquid is applied to the column. (Peristaltic pumb is recomended to keep constant flowing rate.)

It is indispensable, that a flow rate of 1.5 mL/min is not exceeded.

Let the liquid pass the column completely until there is no more sample in the column!

Avoid a complete dehydration of the bed. Rinse the column with 2 mL pure water. Remove residual water using low gas flow or low pressure.

Apply 0.4 mL methanol to the open column by a single-use syringe and wait until the methanol reached the bottom luer-exit of the column. Close the column immediately and incubate it for 5 minutes in order to break the analyte-antibody-binding completely.

After reopening, collect the eluate and press the remaining methanol out of the column into the elutions container using the syringe. Afterwards dilute or concentrate the eluate for the respective requirements and measure it using HPLC.

HPLC Conditions

Ochratoxin A

HPLC: Dionex Ultimate 3000 isocratic
Column oven: 40 °C
Separation column: RP EC125/3 Nucleosil 120-3 C-18 (e.g. P/N 10522)
Flow rate: 0.6 mL/min, water/methanol/acetonitrile (40/55/5 (v/v/v)) + 1 % acetic acid
Excitation wavelength: 335 nm
Emission wavelength: 460 nm

Chromatograms →

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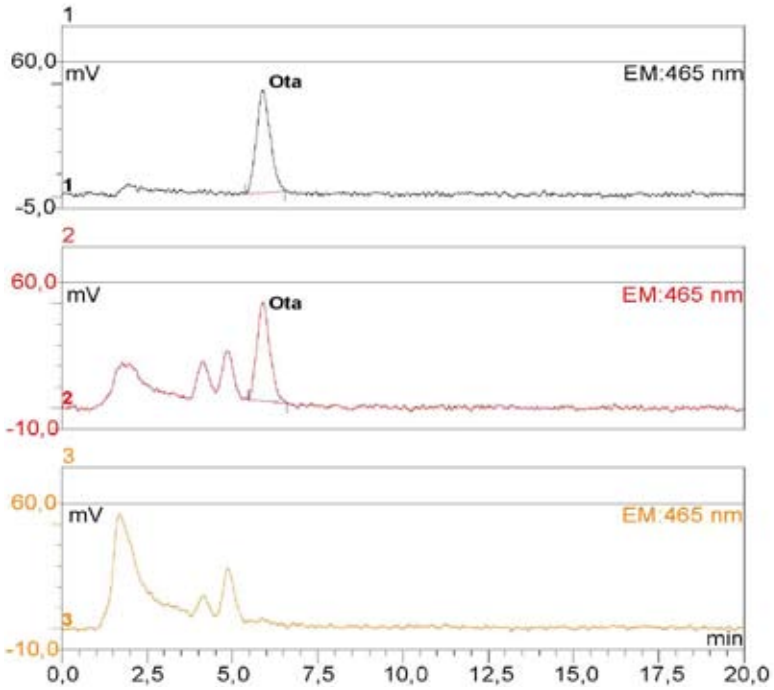
Recovery Rate

Content of Ochratoxin A in Beer	
	Ochratoxin A
Standard*	100
Recovery rate** Beer 1 ppb	100

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

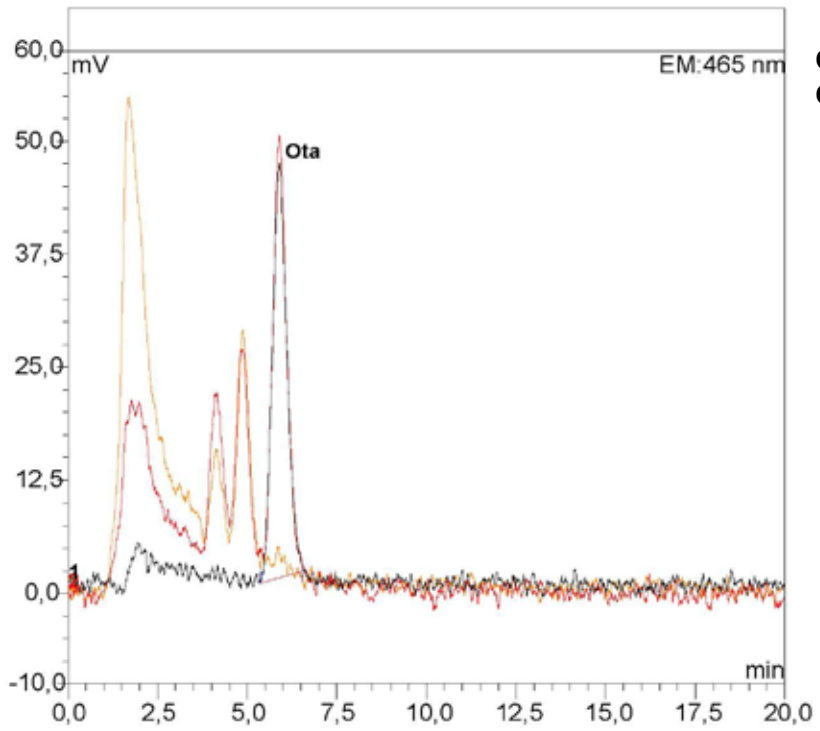
Chromatograms



Standard 1,42 ng/400 µL equivalent to 1 ppb

Beer spiked with 1 ppb OTA

Beer non spiked (blank value)



Ochratoxin A:
Overlay of the cromatograms

These LCTech products were used:

OtaCLEAN SMART,
Immunoaffinity column
for Otatoxin A

P/N 13346 / 13351

HPLC column,
for mycotoxin analysis

P/N 10522

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

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