Matrix of the Month

June, 2013:

Ochratoxin A
in Roasted Coffee

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

5 g sample are extracted with 40 mL (methanol / 3 % NaHCO3, 1/1, v/v).

10 mL of the raw extract are thoroughly mixed with 10 mL dichlormethane for 5 min.

The upper phase is once again mixed with 10 mL dichlormethane for 5 min. Phase separation can be supported by centrifugation at 1000 g for 10 min. The upper phase is filtrated through a platted filter. If phase separation occurs, the upper phase has to be used for the next steps.

3 mL of the filtrated extract are diluted with 72 mL PBS buffer (pH 7.2), the sample now has to be filtered or centrifuged.

The column is opened, let storage buffer leave the column.

50 mL of the diluted raw extract (depending on measurement sensitivity) are added onto the immunoaffinity column OtaCLEAN.

The sample reservoir is rinsed with 10 mL water, this solution is added onto the column. Remaining water can be removed with light gas stream or low-pressure.

1 mL methanol is added onto the open column. Wait until methanol has reached the Luer outlet below. The column is now closed and incubated for 5 min, so that the analyte-antibody bindings can be entirely broken. When the column has been opened again the eluate is collected and eluted with further 1 mL methanol.

Than the extract is concentrated or diluted (depending on requirements) and measured with HPLC.

HPLC Conditions

HPLC: Dionex Ultimate 3000

Column oven: 40 °C

Separation column: Mycotoxin HPLC column EC 120-3 Nucleosil with guard

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

Post column derivatisation with PCX5200, derivatisation reagent sodium hydroxide solution 1M, 40 °C reactor temperature, flow rate 0.3 ml/min

Excitation wavelength: 390 nm

Emission wavelength: 440 nm

Recovery Rate

Content of Ochratoxin A in roasted coffee	
	Ochratoxin A
Standard* 10 ppb	100
Recovery rate** roasted coffee, spiked with 10 ppb	98

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

Chromatograms →

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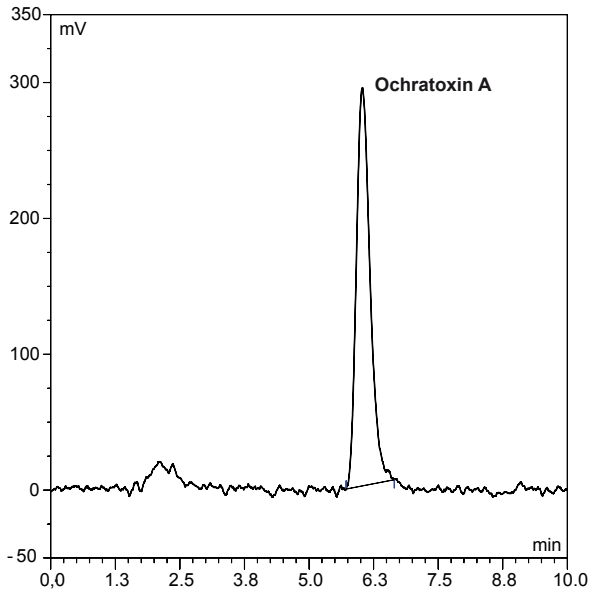
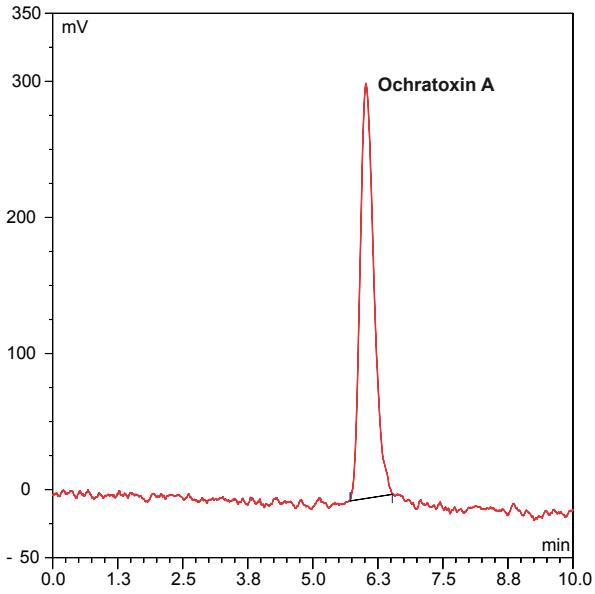
Chromatograms

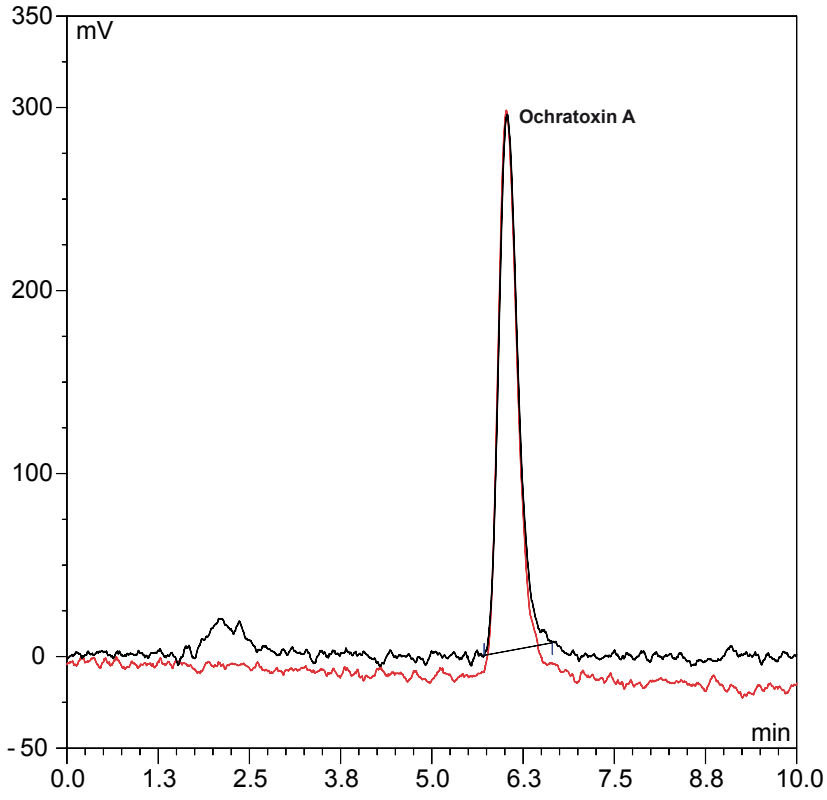
Standard, representing 100 %



Roasted coffee, spiked with 10 ppb toxin



Overlay of both chromatograms



This LCTech product was used:

OtaCLEAN,
Immunoaffinity column
for Ochratoxin A

P/N 10515

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

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