



November 2018

Ochratoxin A in Coffee ~ Manual and Automated ~

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

Sample Preparation

MYCOTOXINS

For a Perfect Start into the Day

A start into the day without a cup of coffee? - „No way!“ - for most Germans. Especially in the cold season, when it is frosty and grey outside, the warmth and the caffeine contained in the coffee ensure that the good mood returns.

If coffee is drunk in moderation, it even has extremely positive effects on people. It stimulates the metabolism, helps against headaches and increases blood circulation in the brain. Therefore after a cup of coffee we are more attentive and concentrated than before.

Automated Clean-up of Ochratoxin A with FREESTYLE SPE

In order to ensure the good quality of coffee further, LCTech supports laboratories worldwide in the field of sample clean-up for food and feed. Especially for the clean-up of ochratoxin A, we offer the immunoaffinity columns OtaCLEAN, which are suitable for both manual and automated processing.

In combination with the LCTech robotic system FREESTYLE SPE, your daily routine tasks can be processed automatically and reliably around the clock - even on weekends. Therefore more time for other important laboratory tasks is rescued.

For the automated clean-up of ochratoxin A in coffee, extract, filter and dilute the coffee according to the processing protocol on the following page. Afterwards, place the samples in the FREESTYLE SPE, equip the racks with the OtaCLEAN immunoaffinity columns, select the method in the software, and start the system - done.



Processing Protocol

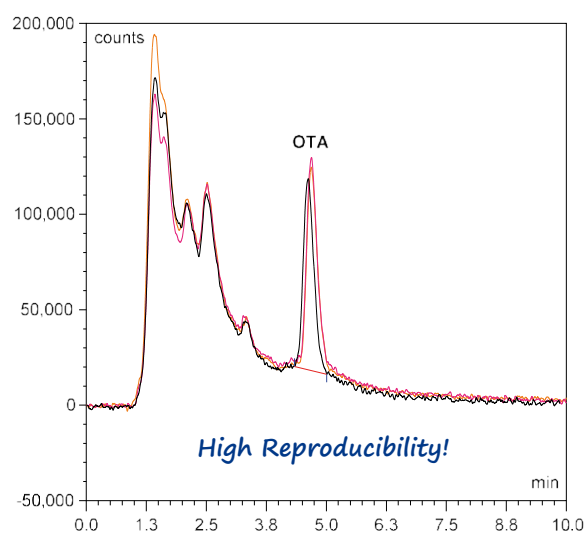
Homogenise 10 g of coffee and add 1 g of sodium chloride. Extract the mixture through 50 mL of methanol/water (80/20 v/v) and add 25 mL of n-hexane in order to remove fat and essential oils. To ensure high extraction efficiencies, continue the extraction for at least 10 minutes.

Filtrate the raw extract and dilute 2 mL of it with 12 mL PBS (contains 8 % Tween20). Load the sample (0.4 g matrix) onto an OtaCLEAN immunoaffinity column and wash the column afterwards with 10 mL of deionised water. Dry the column via a short air flush.

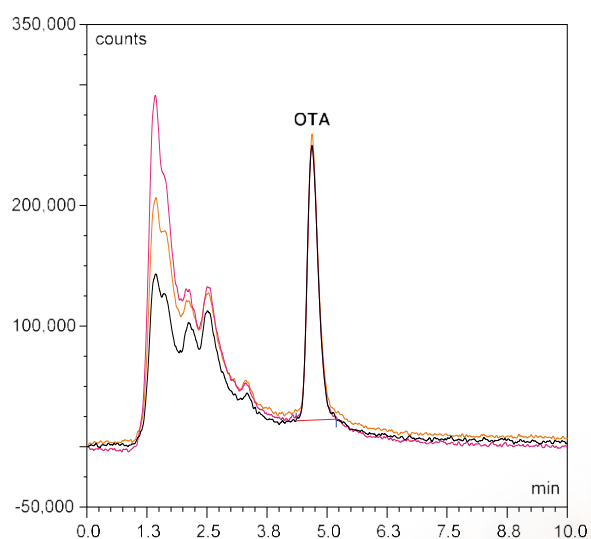
Elute the toxin with 2 mL of methanol. Keep the column bed incubated with methanol for 5 minutes in order to ensure a fully denaturation of the antibodies.

Dilute the sample to eluent conditions and measure it afterwards via HPLC-fluorescence or LC-MS.

Chromatograms



Coffee 2 ppb, cleaned-up via 3 OtaCLEAN columns, represented by the colours black, orange, and red.



Coffee 5 ppb, cleaned-up via 3 OtaCLEAN columns, represented by the colours black, orange, and red.

HPLC-Conditions (Ochratoxin A)

Mycotoxin:	Ochratoxin A
HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	RP C-18 (P/N 10522)
Flussrate:	0.6 mL/min
Flow Rate:	HPLC-Water/Methanol/ Acetonitrile (40/55/5 (v/v/v)) + 1 % Acetic Acid
Flourescence Detection:	without Derivatisation
Excitation Wavelength:	335 nm
Emission Wavelength:	465 nm

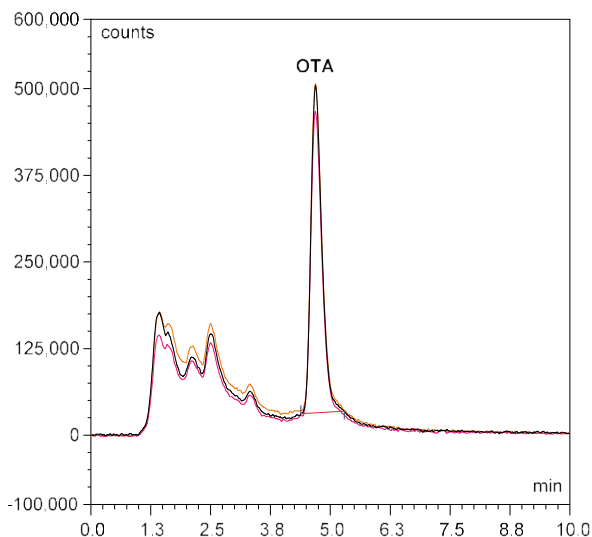
Recovery Rates

Content of Ochratoxin A in Coffee

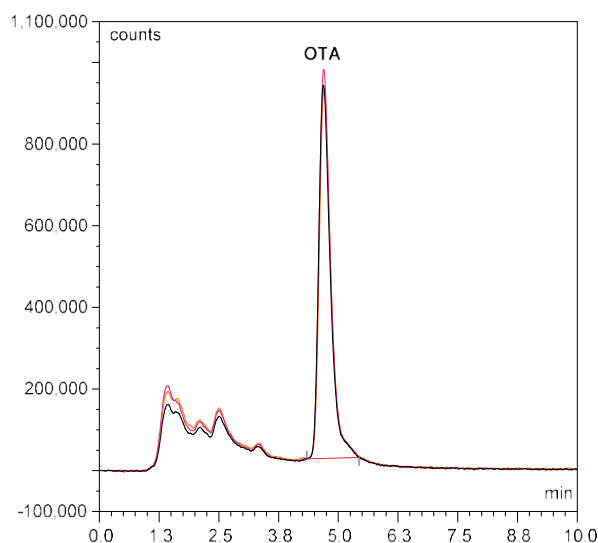
Mycotoxin	Ochratoxin A (n=3)
Standard*	100
Recovery Rate** Coffee, 2 ppb	92 (+/-2)
Recovery Rate** Coffee, 5 ppb	92 (+/-0)
Recovery Rate** Coffee, 10 ppb	95 (+/-1)
Recovery Rate** Coffee, 20 ppb	98 (+/-1)

*Standard is set = 100 %, **Corrected with non-spiked sample /
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

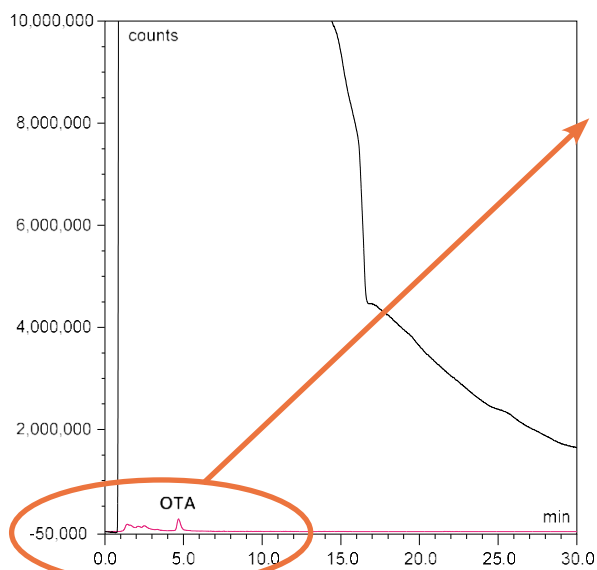
More Chromatograms



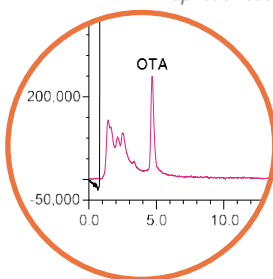
Coffee 10 ppb, cleaned-up via 3 OtaCLEAN columns, represented by the colours black, orange, and red.



Coffee 20 ppb, cleaned-up via 3 OtaCLEAN columns, represented by the colours black, orange, and red.



Black: Coffee 5 ppb, without clean-up;
Red: Coffee 5 ppb, cleaned-up via OtaCLEAN



The sample is so pure that chromatography is possible in only 10 minutes without any interference signals.



Conclusion

The chromatograms show that coffee can be cleaned-up using OtaCLEAN with excellent results and high recovery rates.

The overlapping of the sample cleaned-up with three OtaCLEAN immunoaffinity columns shows the very high reproducibility that can be achieved even with such difficult samples.

The depletion of matrix components demonstrates the selectivity and efficiency of the OtaCLEAN columns, which allow a high measurement sensitivity.

These LCTech products were used:

OtaCLEAN Immunoaffinity Columns for Ochratoxin A
P/N 10515 / 11535

HPLC Separation Column RP C-18
P/N 10522

FREESTYLE SPE Robotic System for Automated
Sample Preparation
P/N 12663, 12668