



Ochratoxin A in Rye Bread: manual or fully automated with FREESTYLE SPE

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 and Analysis

Ochratoxin A is a naturally occurring mycotoxin, which is produced by *Aspergillus* and *Penicillium* species as primary contaminant in various food and feed stuffs. For food and feed analysis and application clean-up purposes, LCTech developed the immunoaffinity column OtaCLEAN.

The columns guarantee best results even at difficult matrices. Particular easy in combination with the FREESTYLE robotic system for fully automated sample preparation, day and night, including weekends.

Automated Processing with FREESTYLE SPE

Any manual SPE method that has proved successfully in your laboratory, can be automated without any problems. With FREESTYLE SPE you can process many different SPE-column formats from 1 to 15 mL. Such as the 3 mL OtaCLEAN immunoaffinity column of LCTech. Extract, filtrate and dilute the rye bread according to the description of the manual processing. Put your samples into the FREESTYLE SPE, equip the racks with OtaCLEAN columns, choose the method from the software and press the start button.

MYKOTOXINS



Immunoaffinity column OtaCLEAN



Especially fast... Especially simple...

- Excellent recovery rates
- No cross-contamination
- Extremely fast and precise processing
- Very simple and intuitive software

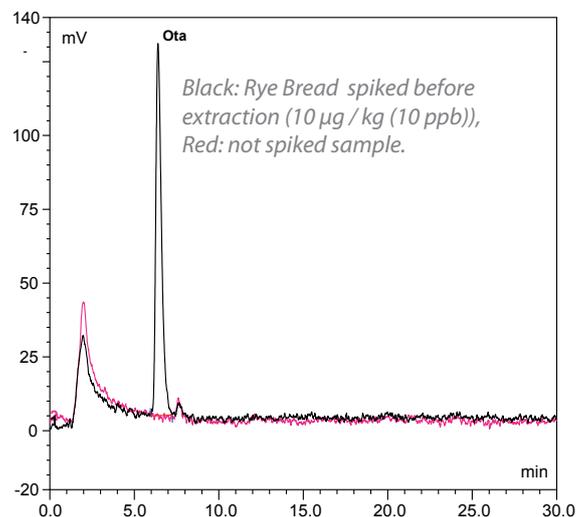
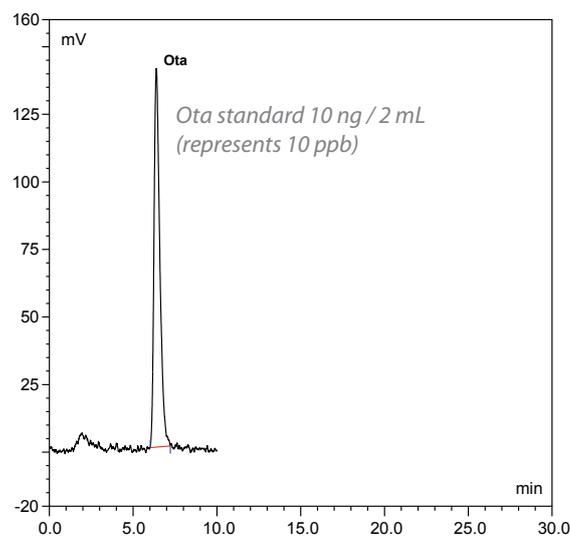
Protocol of Manual Processing

Homogenize 20 g of rye bread and add 2 g sodium chloride. Extract the sample material with 100 mL (methanol/water (80/20 (v/v))) and 50 mL n-hexane for at least 10 minutes. Filtrate the raw extract. You can centrifuge the extract. To facilitate the phase separation, dilute 10 mL with 40 mL PBS. In case of precipitation or turbidity you can remove them by filtration.

Load 25 mL (represents 1 g matrix) onto a 3 mL OtaCLEAN column with a flowrate of max. 2 mL/min. Wash the sample reservoir with 10 mL de-ionized water and load this solution onto the immunoaffinity column.

Dry the column by flushing air through it and eluate afterwards with 2 mL methanol. Take care, that the methanol incubates within the column bed for 5 minutes.

Collect the eluate and dilute it to HPLC conditions of the subsequent analysis.



HPLC-Conditions (Ochratoxin A)

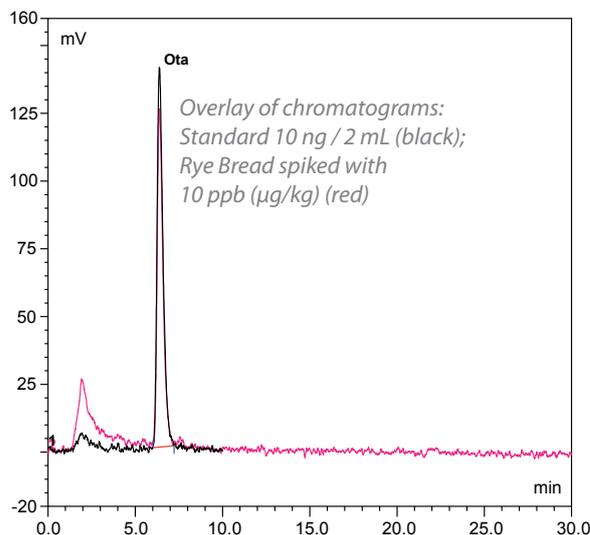
HPLC:	isocratic
Column Oven:	40°
Separation Column:	RP EC 125/3 nucleosil 120-3 C18
Flowrate:	0.6 mL/min
Eluent:	HPLC-water/methanol/acetonitrile (40/55/5) + 1 % acetic acid
Fluorescence Detection:	without derivatisation
Excitation Wavelength:	335 nm
Emission Wavelength:	465 nm

Recovery Rates

Content of Ochratoxin A in Rye Bread

Standard*	100
Recovery Rate** Rye Bread, 10 ppb	88

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



These LCTech products were used:

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

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