



November 2016

## Ochratoxin A in Trail Mix - 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](mailto:mycotoxins@LCTech.de)

### Sample Preparation

MYCOTOXINS

#### Trail Mix („Student Feed“)

Since the 17th century „student feed“ is a common name for the mix of nuts and dried fruit in Germany. Though the trail mix is extremely high of calories, it also provides heaps of energy, vitamins and minerals and thus is a very popular snack for young and old. The risk of contamination in such foodstuffs arises from storage and pre-products which may be contaminated with mold.

#### Immunoaffinity Columns OtaCLEAN for Ochratoxin A

Ochratoxin A is a naturally occurring mycotoxin, which is produced by various *Aspergillus* and *Penicillium* species as primary contaminant in various food and feed stuffs. This year the rapid alert system RASFF has found high concentrations of ochratoxin A in nuts and aflatoxins in nut-raisin mixes. For sample preparation and analysis of ochratoxin A in food and feed LCTech developed the immunoaffinity columns OtaCLEAN. The columns possess a very high matrix tolerance and are able to bind ochratoxin A with a very high specificity. They are available in a convenient 3 mL format as well as in a 1 mL format. For even faster automated processing we provide OtaCLEAN SMART columns with a size of only 3 cm.



#### Automated Processing via FREESTYLE SPE

Clean-up with OtaCLEAN can be done either manually or automatically with FREESTYLE SPE. The robotic system takes over routine laboratory tasks around the clock and even at weekends.

The system processes unattended, yet reliably, various applications for mycotoxin analysis. Extract, filtrate and dilute the trail mix 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.

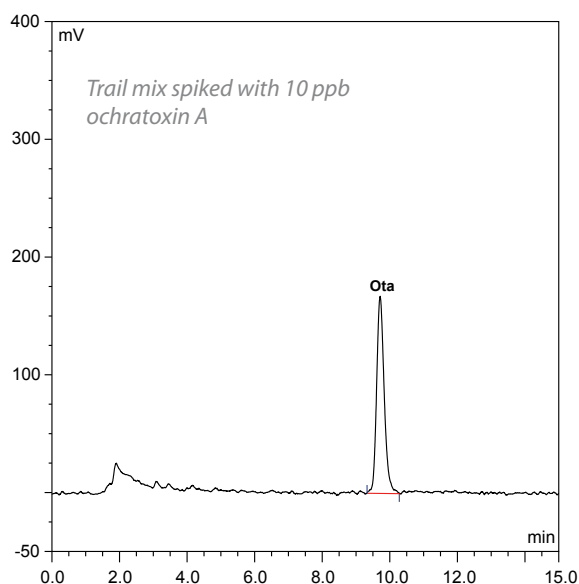
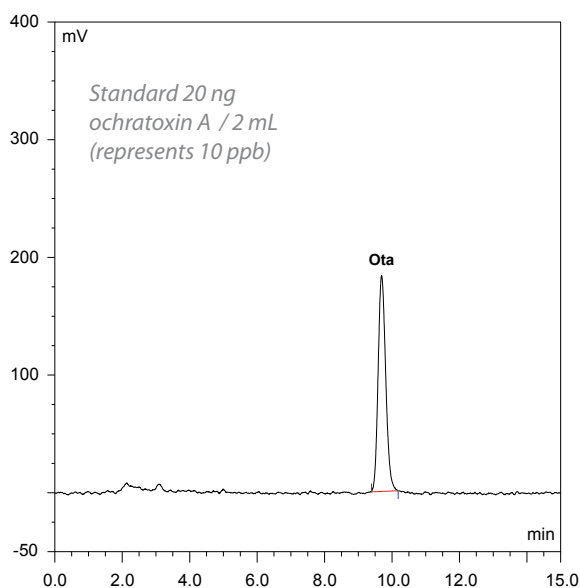
## Protocol of Manual Processing

Homogenise 10 g of trail mix (nut-raisons mix) and add 1 g of sodium chloride. Extract the sample with 50 mL methanol/water (80/20 (v/v)) and dilute 15 mL with 60 mL PBS.

Filtrate the diluted sample again through a glass fiber filter, in order to remove turbidities and precipitations. Load 50 mL of the filtrated sample (represents 2 g matrix) onto the immunoaffinity column OtaCLEAN with a maximum flow rate of 2mL/min. Wash the column with 10 mL deionized water, which was used for rinsing of the sample reservoir.

Dry the column by flushing air through it and add afterwards 2 mL methanol to elute the toxin. Keep in mind that the column bed is incubated with methanol for at least 5 minutes in order to ensure the complete denaturation of the antibodies.

## Chromatogramme



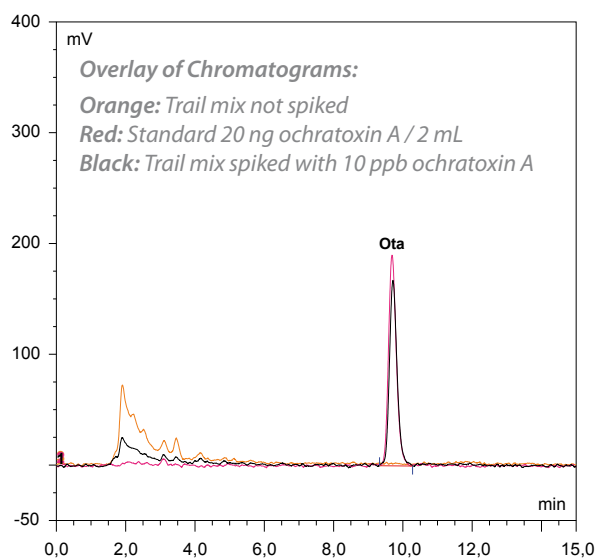
## HPLC-Conditions (Ochratoxin A)

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

## Recovery Rates Content of Ochratoxin A in Trail Mix

| Mycotoxin                                    | Ochratoxin A |
|--|--------------|
| <b>Standard*</b>                             | 100          |
| <b>Recovery Rate**<br/>Trail mix, 10 ppb</b> | 83           |

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



## These LCTech products were used:

OtaCLEAN, 3 mL  
Immunoaffinity Columns for Ochratoxin A  
P/N 10515 / 11535



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