



June 2017

## Ochratoxin A in Rice Comparison: Manual and Automated Processing

*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

#### Ochratoxin A

Cultivation and storage of food and animal feed may contribute to the spread of mold which produce mycotoxins - toxic secondary metabolites. Their consumption can lead to serious health damage in both humans and animals. Ochratoxin A is a naturally occurring mycotoxin, which is produced by various *Aspergillus* and *Penicillium* species as primary contaminant under bad storage conditions in various food and feed stuffs. It can be found in numerous types of grain like rice, dried fruits, or nuts.

#### Automated Processing via FREESTYLE SPE

Sample clean-up via SPE-column belongs to the protracted and monotonous routine tasks in any analytical laboratory - however, this job demands highest concentration because the samples should not get mixed up. This is an ideal process step for automated processing. The FREESTYLE SPE has unique possibilities for sample preparation using solid phase extraction (SPE). Diligently day and night, the longest running applications in mycotoxin analysis will be accurately processed. The FREESTYLE SPE can be used for all types of mycotoxins columns. Elution can be conveniently carried out in a 2 mL or 5 mL flask, such that the eluted volume can be adjusted quickly to a precise value.

Each already approved manual SPE method in the laboratory can be automated in a quick and easy manner. The application fields are wide: from mycotoxin and environmental analysis up to forensic applications and doping control samples.

*The next generation of automated sample preparation.  
Flexibel, precise, modular.*



## Preparatory Steps for the Following Processing

Extract 20 g homogenised rice with 100 mL methanol/water (80/20 (v/v)). The extraction should be performed 10 minutes. Filtrate the raw extract through a glass fiber filter (Whatman GF/A), in order to remove turbidities and precipitations.

## Manual Processing

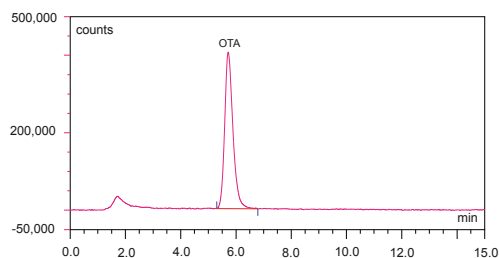
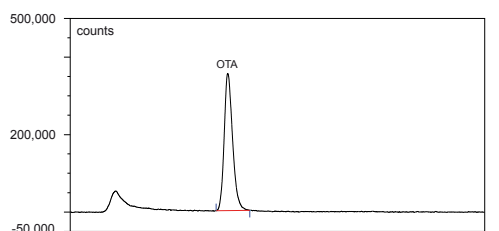
Dilute 12 mL sample with 48 mL PBS and load 25 mL of the sample (equates to 1 g matrix) onto the OtaCLEAN immunoaffinity column. Wash the column with 10 mL deionized water and dry it afterwards by flushing air through it.

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

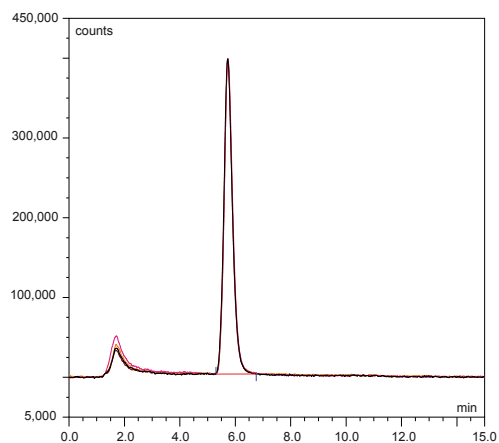
## Automated Processing via FREESTYLE SPE

For automated processing put the sample in the robotic system, parameterize the method with a few mouse clicks in the software and start the system. You can save the method in the system and call it up or you can adapt it anytime for other applications.

## Chromatograms



Black: Manual processing (rice 10 ppb)  
Red: Automated processing with FREESTYLE SPE (rice 10 ppb)



Overlay of 4 chromatograms, automated processed via FREESTYLE SPE

## 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 (60/30/15 (v/v/v)) + 1 % acetic acid
<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 Rice

Ochratoxin A	Automated	Manual
<b>Standard*</b>	100	100
<b>Recovery Rate** Rice, 10 ppb (n = 4)</b>	84 ± 3	87 ± 2

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

## In Comparison

The recovery rates and the chromatogram of manual and automated processing show, that the results, achieved by the robotic system FREESTYLE SPE, are reliable and highly reproducible. The clean-up of ochratoxin A can thus be automated easily.

Every manual method can be easy transferred to automation - you only need a few clicks in the software of the system. Your samples can be processed unattended, reproducible and around the clock.

## These LCTech products were used:

OtaCLEAN,  
Immunoaffinity Columns for Ochratoxin A  
P/N 10515

Separation Column RP C18  
P/N 10544

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