



September 2018

## Ochratoxin A in Ras el Hanout (Spice Mixture) ~ 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

#### Ras el Hanout

The spice mixture from North Africa consists of various ingredients. Depending on the production, it contains about 25 different spices, including for example nutmeg, cinnamon, curcuma, ginger, or aniseed. The mixture contains sweet as well as spicy and bitter flavours. With its typical oriental taste Ras el Hanout is ideal for dishes with couscous, rice, or millet, but also for seasoning meat, fish, and dips made of cheese and yoghurt.

The flavour is best preserved if the spice mixture is stored cool, dry, and light protected. If stored incorrectly, i.e. too moist, moulds can occur, which, as in the case of our September matrix, produce the toxin ochratoxin A. However, the precursors of Ras el Hanout may already contain these toxins and it is almost impossible for the consumer to recognize this contamination.

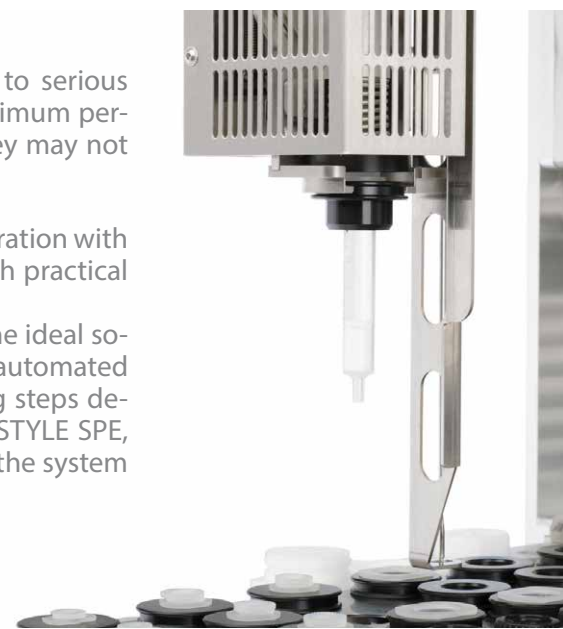
#### Ochratoxin A in Food and Feed

The consumption of food and feed contaminated with moulds can lead to serious health problems for humans and animals. Therefore, legislation has set maximum permitted levels for mycotoxins. If foodstuff exceed these maximum levels, they may not be imported.

LCTech offers a comprehensive product range for all aspects of sample preparation with the aim of making your daily work in the laboratory considerably easier with practical and cost-effective consumables and automated systems.

Especially for the clean-up of ochratoxin A in food and feed, LCTech offers the ideal solution with the immunoaffinity columns OtaCLEAN in combination with the automated robotic system FREESTYLE SPE. Simply carry out the preparatory processing steps described on the following page. Afterwards position the sample in the FREESTYLE SPE, parameterize the method in the software with a few mouse clicks, and start the system - done.

Read more at: [www.LCTech-online.com](http://www.LCTech-online.com)



## Preparatory Processing Steps

Homogenise 10 g of Ras el Hanout, extract it with 100 mL of methanol/water (80/20 (v/v)) and add 50 mL n-hexane in order to remove fat and essential oils. Carry out the extraction for 20 - 30 minutes. Filtrate the extract through a fibre filter to remove precipitations.

Centrifuge the filtrate at 3000 x g in order to achieve a phase separation between the n-hexane and the methanolic phase. Use the methanolic (lower) phase for further processing.

## Manual Processing

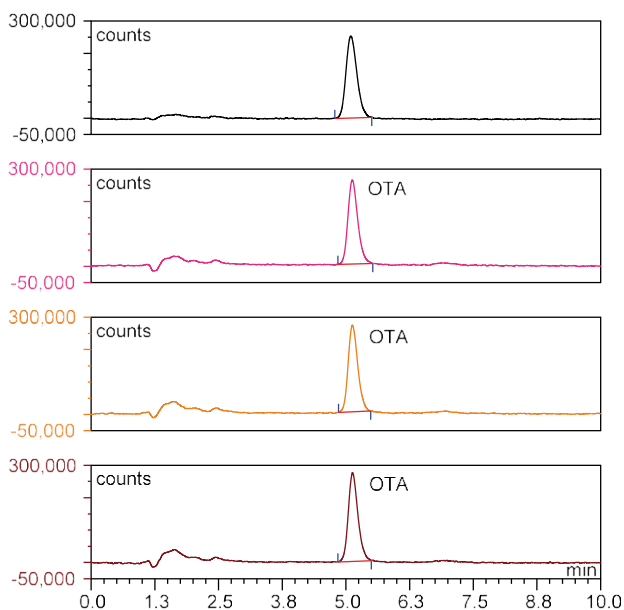
Dilute 2 mL of the sample with 12 mL of PBS (contains 8 % Tween). Load 14 mL of the sample (represents 0.2 g matrix) onto an immunoaffinity column OtaCLEAN. Wash the sample reservoir with 2 x 5 mL of deionised water and load this solution also onto the column.

Dry the column with a short flush of air and elute the toxins with 2 mL of 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.

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

## Chromatograms

In order to show the reproducibility, 3 extracts were cleaned-up and examined chromatographically:



**Black** = Standard 2 ng / 2 mL (10 ppb)

**Pink, Orange, Brown** = 3 samples cleaned-up manual (10 ppb)

## HPLC-Conditions

(Ochratoxin A)

<b>Mycotoxin:</b>	<b>Ochratoxin A</b>
<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 (40/55/5 (v/v/v)) + 1 % acetic acid
<b>Fluorescence Detection:</b>	without derivatization
<b>Excitation Wavelength:</b>	335 nm
<b>Emission Wavelength:</b>	465 nm

## Recovery Rates

Content of Aflatoxin B/G in Ras el Hanout

<b>Ochratoxin A</b>	<b>OTA</b>
<b>Standard*</b>	100
<b>Recovery Rate** Ras el Hanout, 10 ppb</b>	92

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



Immunoaffinity Columns OtaCLEAN

## These LC Tech products were used:

OtaCLEAN,  
Immunoaffinity Column for Ochratoxin A  
P/N 10515 / 11535

FREESTYLE SPE, Robotic System  
for Automated Sample Clean-up  
P/N 12663 / 12668