Matrix
of the Month

LC Tech

February 2019
Ochratoxin A in Coconut Chips
~ 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

Coconut Chips

The coconut represents for most people holiday – beach, sea, sun and palm tree in a foreign exotic country. Besides, the tropical drupe is also a healthy all-rounder. The white, aromatic tasting fruit flesh is not only freshly edible, it can also be dried to chips and rasps. The high content of dietary fibre, omega-6- fatty acids and proteins makes coconut chips to a healthy snack alternative.

Before importing coconut to EU, regular food controls take place, because the fruit is grown and drying in tropical countries. As a result of incorrect storage or processing process, unwanted mould formation can occur, resulting in an excessively high mycotoxin concentration, which is highly regulated in food and feed stuff.

In order to comply with the high food standards, strict legal regulations for the permissible content of mycotoxins apply throughout the EU. If the content is too high, the goods will be rejected.

Clean-up of Ochratoxin A in Food and Feed

The challenge many laboratories have to face nowadays is to be able to process many samples as quickly as possible. To facilitate this, LCTech has developed the 3 cm OtaCLEAN SMART columns in addition to the 3 mL OtaCLEAN immuno-affinity columns.

OtaCLEAN SMART columns convince not only by their small size and a lower price, but also by a reduced solvent consumption, shorter processing time and excellent recovery rates in comparison to the 3 mL columns. Both columns have a very high matrix tolerance and are able to bind ochratoxin A highly specifically. They are suitable for both, manual and automated processing, e.g. with the LCTech robotic system FREESTYLE SPE or the FREESTYLE ThermELUTE™.

Processing on FREESTYLE ThermELUTE™: Gripper with adapter takes the SMART column

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Processing Protocol

Homogenise 20 g of coconut chips and add 2 g of sodium chloride. Extract the mixture through 50 mL of methanol / water (80/20 (v/v)) and add 50 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.
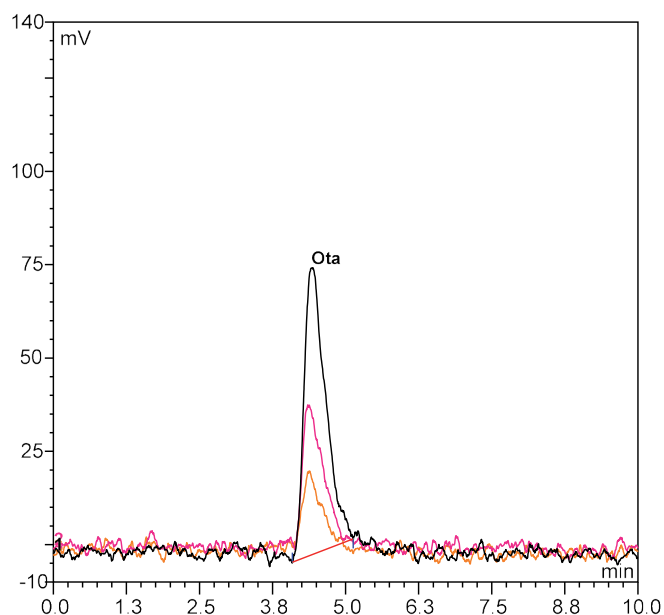
Filtrate the raw extract and dilute 2 mL of the n-hexne freephase with 12 mL PBS (contains 8% Tween20). Load 14 mL sample onto an OtaCLEAN immunoaffinity column and wash the column afterwards with 10 mL of deionized water. Dry the column by a short air flush.

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.

By using the OtaCLEAN SMART column, load 2.8 mL sample onto the column. Wash the column afterwards with 2 mL of deionized water and dry it by flushing air through it. For the SMART column, elute Ochratoxin A with 400 µL methanol.

Keep the column bed incubated with methanol for 5 minutes to ensure a fully denaturation of the antibodies.

HPLC-Conditions
(Ochratoxin A)

| Mykotoxin	Ochratoxin A
HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	RP C-18 (P/N 10544) oder RP EC 125/3 nucleosil 120-3 C18
Flow Rate:	0.6 mL/min
Eluent:	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

Chromatograms

Orange: Standard 5 ppb
Red Standard 10 ppb
Black: Standard 20 ppb

Recovery Rates
Content of Ochratoxin A in Coconut Chips

| Mykotoxin	Ochratoxin A
Standard*	100
Recovery Rate** Coconut Chips, 20 ppb (OtaCLEAN)	92
Recovery Rate** Coconut Chips, 10 ppb (OtaCLEAN)	98
Recovery Rate** Coconut Chips, 5 ppb (OtaCLEAN)	93
Recovery Rate** Coconut Chips, 20 ppb (OtaCLEAN SMART)	87
Recovery Rate** Coconut Chips, 10 ppb (OtaCLEAN SMART)	91
Recovery Rate** Coconut Chips, 5 ppb (OtaCLEAN SMART)	89

*Standard is set = 100 %, **Corrected with non-spiked sample /
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)
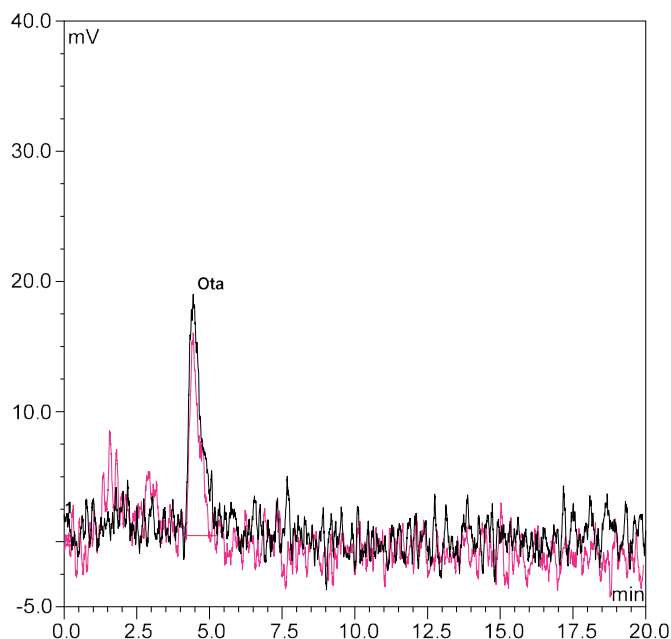
Black: 5 ppb Coconut chips cleand-up with OtaCLEAN
Red: 5 ppb Coconut chips cleand-up with OtaCLEAN SMART
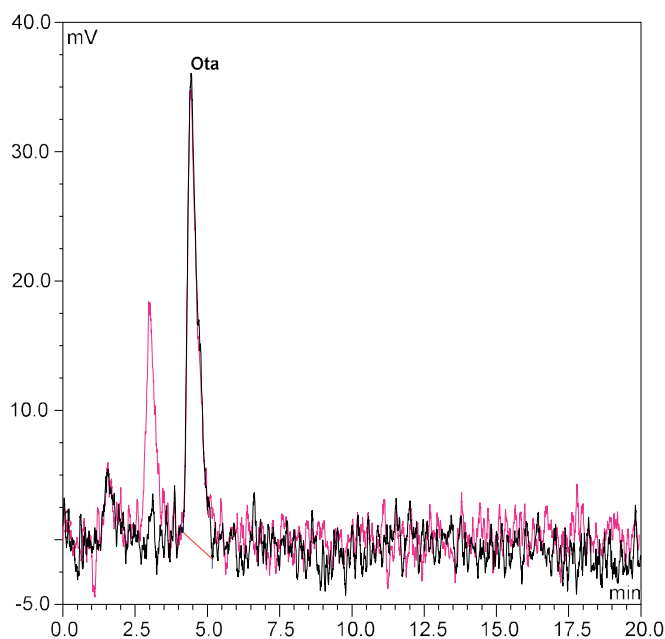
Black: 10 ppb Coconut chips cleand-up with OtaCLEAN
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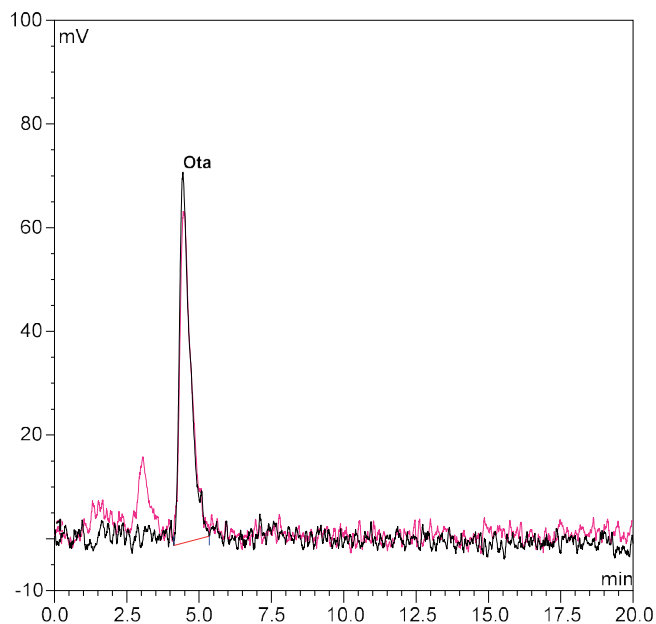
Black: 20 ppb Coconut chips cleand-up with OtaCLEAN
Red: 20 ppb Coconut chips cleand-up with OtaCLEAN SMART

OtaCLEAN SMART Column with accessories for manual processing

Small + Fast + Economical = Clever & SMART

By using the OtaCLEAN SMART column, more than 80 % of the solvents can be saved by extraction, dilution, washing and elution. As a maximum of only 400 µL is used for elution, the processing time is further reduced. This increases the total sample throughput per week. As the chromatograms and very good recovery rates show, the results are absolutely comparable to the OtaCLEAN column in 3 mL format. The only difference: small, fast & economical!

These LCTech Products were used:

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

OtaCLEAN SMART Immunoaffinity Column for Ochratoxin A

P/N 13346 / 13351

HPLC Separation Column RP C-18
P/N 10522

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

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