Matrix of the Month

October 2020

Ochratoxin A in Liquorice
~ 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: info@LCTech.de

Sample Preparation

MYCOTOXINS

Liquorice

Liquorice (bot.), also known as real liquorice, belongs to the legume family. By extracting the root and adding various ingredients such as sugar syrup or flour, the typical sweetness is created. Liquorice is not only used as a stimulant, as in sweets or drinks, but is also used in medicine.

Even in ancient Rome, people knew about the health-promoting effects of liquorice. Liquorice was used to treat throat and stomach ailments. Today, the Middle East is one of the classic growing regions for genuine liquorice. Licorice as a confectionery is consumed worldwide. Especially the Dutch have a special preference for licorice, so called „Drop".

Ochratoxin A in Difficult Matrices

Ochratoxin A is a naturally occurring mycotoxin produced by moulds of the genera Aspergillus and Penicillium as primary contamination. Ochratoxin A is also found in liquorice due to its high liquorice (Glycyrrhiza glabra) content. Nearly all liquorice (bot.) has levels of ochratoxin A.

The essential oils and vegetable colourings, as well as the high sugar content of liquorice often are challenges during sample preparation. LCTech offers with the immunoaffinity columns OtaCLEAN and the robotic system FREESTYLE SPE an efficient sample preparation. More samples in less time and high cost savings.

Any manual SPE method that has proven itself in your laboratory can be directly transferred to the system. Already created methods can be saved and reused, but also modified. Even with difficult matrices, such as liquorice, show very good recovery rates.

FREESTYLE SPE with Immunoaffinity Column OtaCLEAN

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Processing Protocol

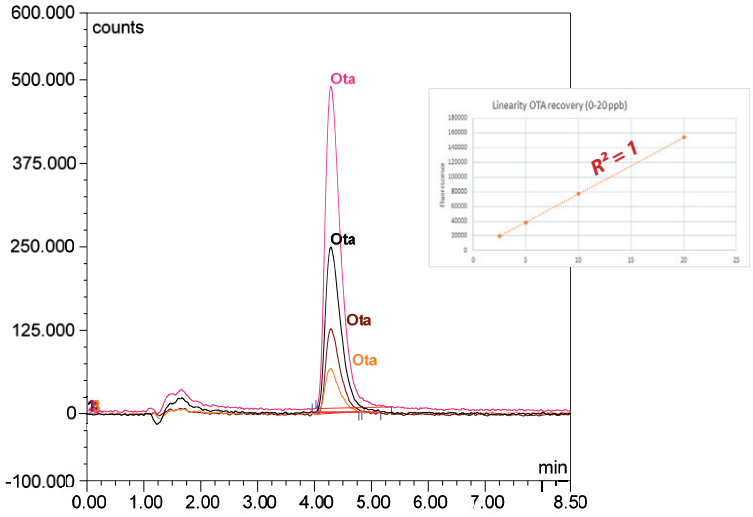
Homogenise 20 g of liquorice and add 2 g of sodium chloride. Extract the sample by 100 mL methanol/water (80/20 (v/v)) and add 50 mL of n-hexane in order to remove fat and essential oils for at least 20 minutes.

Filter the raw extract and dilute 2 mL of the n-hexane free phase with 12mL PBS (contains 8 % Tween). Load the sample onto the immunoaffinity column OtaCLEAN. Ensure that the flow rate does not exceed 2mL/min.

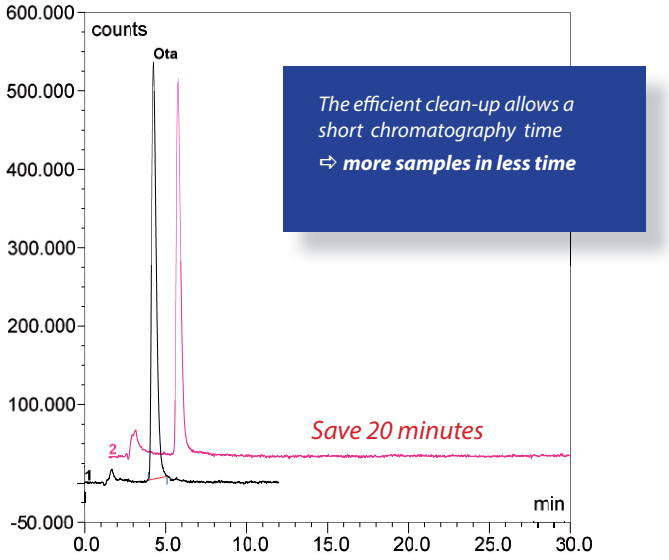
Then rinse the sample reservoir with 5 mL deionised water and load the washing solution onto the column. Wash the column again with 5 mL deionised water. Afterwards, you can remove any residual liquid from the column with a short flush of air.

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

Chromatograms



Red 20 µg/Kg, black 10 µg /Kg, brown 5 µg/Kg, orange 2.5 µg/Kg



The efficient clean-up allows a short chromatography time

⇨ ***more samples in less time***

Black: Standard 20 µg/Kg

Red: Liquorice 20 µg/Kg, cleaned-up with Immunoaffinity Column OtaCLEAN

HPLC-Conditions

Ochratoxin A

HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	EC125/3 Nucleosil 120-3 C-18
Flow Rate/ Eluent:	0.6 mL/min; 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

Recovery Rates

Content of Ochratoxin A in liquorice

Mycotoxin:	**Ochratoxin A**
Standard*	100
Recovery Rate Liquorice, 10 ppb**	89
Recovery Rate Liquorice, 20 ppb**	88

*** Standard was set = 100% , ** Corrected with non-spiked sample / The results are in accordance with the performance specifications of the EC 401 / 2006 (section 4.3.1).**

These LCTech Products were used:

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

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

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