Matrix of the Month

LCTech wishes you Merry Christmas!

December 2017

Aflatoxins B/G in Marzipan ~ 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

Marzipan

One thing should not be missing in the pre-Christmas period: sweets! Marzipan though, the noble sweetness made of almonds, sugar and rose water, is especially hard to resist. But as tasty it is – as dangerous it can be. Due to incorrect or too long storage of almonds contained in the marzipan paste, they are often exposed to highly poisonous moulds, so called aflatoxins. These toxins are not immediately recognisable by smell, taste or with the naked eye. Particularly for the import from third countries the implementing regulation of the European commission, (EC) No. 1152/2009 set intensive sampling and investigations in order to control the high risk of aflatoxin contamination.

Automated Mycotoxin Analysis

Unattended, Reliable and Around-the-Clock with the Robotik System FREESTYLE SPE

Diligently during day, night and at weekends, the robotic system FREESTYLE SPE processes even the longest running applications in mycotoxin analysis accurately. LCTech's approach to the handling of SPE columns is as simple as it is effective. The column is received by the z-axis of the robot, gripped air and liquid tight, and is connected with the injection pump and valve system via an integrated tube. This unit ensures the uniqueness of the system and enables the SPE column to be moved to any place on the platform.

Each manual SPE-method which has already established in the laboratory can be automated in quick and easy manner. Extract, filtrate and dilute the marzipan according to the protocol of manual processing. Equip the racks with the immunoaffinity columns AflaCLEAN, configure the required method in the easy to operate software and press the start button.

Besides the LCTech immunoaffinity and clean-up columns, the FREESTYLE SPE can be used for all types of mycotoxin columns of leading manufactures.

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Protocol of Manual Processing

Homogenise 20 g of marzipan and add 2 g of sodium chloride. Extract the mixture through 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane in order to remove fat and oil. Carry on the extraction for 20 minutes.

Filtrate the raw extract and dilute 7 mL of it with 43 mL PBS. Filtrate the sample again through a glass fiber filter to remove turbidities and precipitations. Load 50 mL of the sample with a max. flow rate of 2 mL/min. onto the immunoaffinity column AflaCLEAN. Wash the sample reservoir afterwards with 2 x 5 mL deionised water and load this solution also onto the column.

Dry the column with a short flush of air and elute it 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 and release of the toxins.

Dilute the eluate to HPLC conditions and measure it afterwards via fluorescence or LC-MS.

Chromatograms

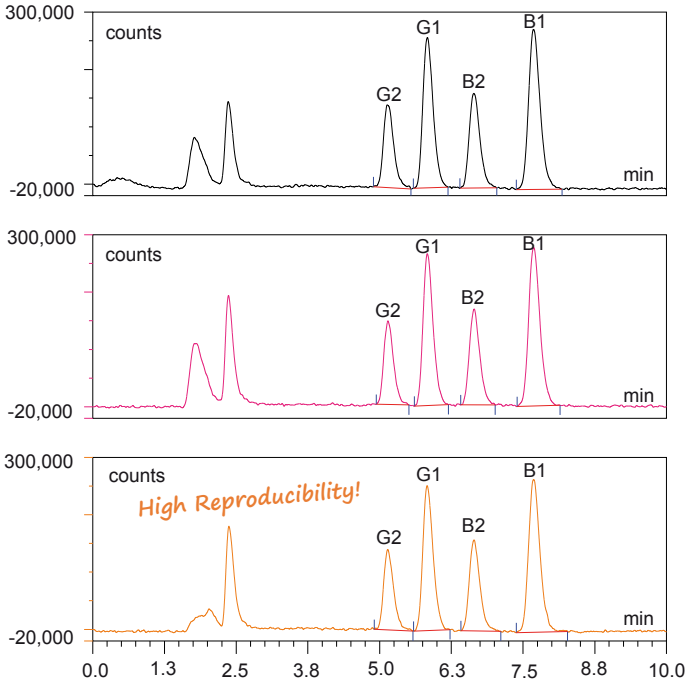

Figure of 3 chromatograms spiked with 10 ppb and cleaned-up with immunoaffinity columns AflaCLEAN

Conclusion

The chromatograms show that even with quite complex matrices, like marzipan, clean-up with AflaCLEAN columns results in good chromatographic resuclts with excellent recovery rates.

The high purity of the sample, enables a chromatography within less than 10 minutes, which is particularly interesting for high throughput laboratories.

HPLC-Conditions
(Aflatoxins B/G)

Mycotoxin:	**Aflatoxins B/G**
HPLC:	isocratic
Column Oven:	36 °C
Separation Column:	RP C-18 (P/N 10522)
Flow Rate:	1.2 mL/min
Eluent:	HPLC-water/ methanol/acetonitrile (60/30/15 (v/v/v))
Fluorescence Detection:	Derivatisation with UVE Photochemical Reactor
Excitation Wavelength:	365 nm
Emission Wavelength:	460 nm

Recovery Rates
Content of Aflatoxins B/G in Marzipan

Aflatoxins	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate Marzipan, 10 ppb**	89	93	84	90

*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:

AflaCLEAN, Immunoaffinity Column
for Aflatoxins B/G
P/N 10514 / 11721

HPLC Separation Column RP C-18
P/N 10522

UVE, Photochemical Reactor
P/N 10519

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