



August 2017

Aflatoxins B/G in Peanut Butter ~ 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

The History of Peanut Butter

Peanut butter is an invention of Dr. John Harvey Kellogg from the 19th century, who also invented the cornflakes at that time. He pureed peanuts in order to obtain a nutritious food for patients without teeth. Today about half of the peanut harvest of the USA goes into the production of peanut butter. As this is still not enough, further tons of nuts and finished products are imported. Despite the high calorie content (90 calories per tablespoon!), peanut butter is healthy: it contains plenty of vitamin E and H and its also a very good energy supplier. In the USA it is used as a classic bread spread, but also peanut butter biscuits, brownies or cakes are very popular.

However, for the import of food and feed, there are strict EU-wide-regulations for the permissible content of mycotoxins. Thus, an effective and significant analysis is essential. In 2016 alone, 177 exceedances of the limits for aflatoxins B/G in peanuts were found followed by the consequence of an import ban. [Source: RASFF-Portal]

Automated Processing with FREESTYLE SPE

The number of SPE methods is virtually innumerable. The individual method requirements couldn't be more different. Yet almost all methods can be automated using the FREESTYLE SPE - without compromise.

Many different possible applications arise through the very flexible sample loading: from large-volume of up to 100 mL in mycotoxin analysis to only a few μL e.g. in forensics. These tasks are processed reliable and consistently during day, night, and over the weekend.



Protocol of Manual Processing

Homogenise 20 g of peanut butter and add 2 g of sodium chloride. Extract the sample with 100 mL methanol/water (80/20 (v/v)) and 50 mL of n-hexane in order to remove fat and oils. The extraction should be conducted for 20 - 30 minutes.

Centrifuge the extract for the phase separation between the aqueous and the n-hexane phase with 2000 x g for 10 minutes. Dilute 10.5 mL of the aqueous (lower) phase with 64.5 mL PBS-buffer. In case of precipitations filtrate the sample with a glass fiber filter.

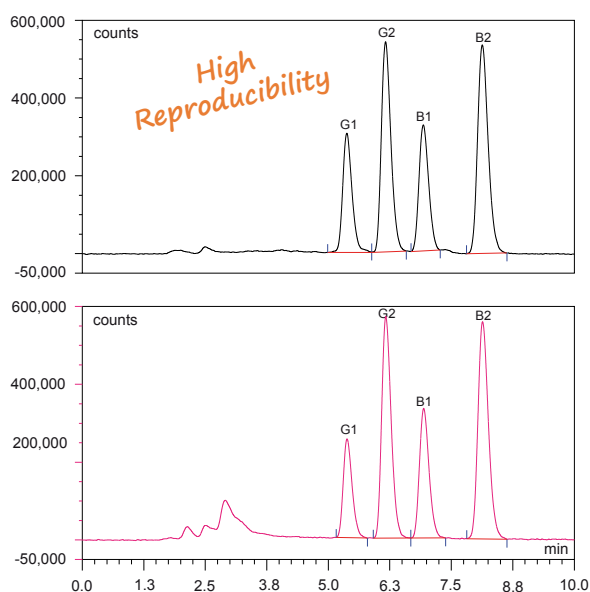
Load the extract onto a AflaCLEAN immunoaffinity column. Wash the sample reservoir afterwards with 2 x 5 mL deionised water and load this solution also onto the IAC-column.

Dry the column and elute it with 2 mL methanol. Keep in mind, that the methanol incubates for 5 minutes into the column bed, in order to dissolve the antibody toxin bond completely. Dilute the sample to HPLC conditions and measure it afterwards.

Excellent Results

The table on the right shows the good recoveries and the high correlation between toxin loading and recovery achieved with the AflaCLEAN column. Thus, the immunoaffinity column is able to achieve comparable recoveries in a very wide measuring range (0.4 - >20 ppb toxin loading).

The following chromatograms show how efficient the IAC-column achieves an accumulation of the Aflatoxins B/G and simultaneously removes contaminants, which could have a negative effect to the analysis. So you get a precise chromatogram without any interfering signals.



Black: Standard 14ng/2 mL (10 ppb), Red: Peanut butter spiked 10 ppb

HPLC-Conditions

(Aflatoxin B/G)

HPLC	isocratic
Column Oven	36 °C
Separation Column	RP C-18 (P/N 10544)
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 Aflatoxin B/G in Peanut Butter

Aflatoxin B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rates** Peanut Butter				
0,4 ppb Total Toxin (0,1 ppb B1)	103	100	93	79
Error (%)	5	2	3	3
1 ppb Total Toxin (0,4ppb B1)	94	93	89	74
Error (%)	5	2	4	3
2 ppb Total Toxin (0,8 ppb B1)	95	91	95	78
Error (%)	3	3	3	1
4 ppb Total Toxin (1,6 ppb B1)	91	92	91	73
Error (%)	6	6	6	3
5 ppb Total Toxin (2 ppb B1)	103	104	102	83
Error (%)	4	4	4	3
10 ppb Total Toxin (4 ppb B1)	97	98	97	75
Error (%)	4	4	4	4
20 ppb Total Toxin (8 ppb B1)	98	99	97	76
Error (%)	5	5	6	4

*Standard is set = 100 %, **Corrected with non-spiked sample / The middle 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

UVE, Photochemical Reactor
P/N 10519

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