



August 2019

Aflatoxin B/G in Peanuts ~ 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

Peanuts

Is the peanut a real nut? At least it is part of the name of the fruit. In the true sense, the peanut is a legume. Thus, peanuts are related to beans and peas. In contrast to real nuts, the fruit wall of peanuts is not woody, but only a pod. The proportion of omega-3 fatty acids in legumes is also significantly lower than in nuts. Peanuts are not only tasty and nutritious, they are also indispensable in the food and feed industry.

Since peanuts in their raw state cause flatulence and abdominal pain, you won't find any raw peanuts in German supermarkets, only cooked or roasted peanuts. During further processing of the peanuts, mycotoxins may be produced under incorrect storage conditions, which may be toxic to humans in a too high proportion. For this reason, strict import controls are carried out by the European Union.

Fully Automated Mycotoxin Analysis with FREESTYLE ThermELUTE™

For many laboratories, the challenge is to analyse as many samples as possible in less time. To facilitate this task, LCTech has developed the **AflaCLEAN SMART** column in addition to the **AflaCLEAN immunoaffinity column**.

By using the 3.5 cm small SMART column, a particularly high sample throughput of 500 samples per week can be achieved. The **FREESTYLE ThermELUTE™** also enables fully automated mycotoxin analysis with results so sensitive that it is easy to measure far below the limit values.

Each sample can be processed with the AflaCLEAN SMART column in FREESTYLE ThermELUTE™. This ensures 100 % performance for each sample and reliably prevents cross-contamination.



ThermELUTE™ gripper injects SMART column

Processing Protocol

Homogenise 20 g of peanuts and add 1 g of sodium chloride. Extract the mixture with 100 mL methanol/water (80/20 (v/v)) and add 50 mL n-hexane in order to remove fat and oil. Filtrate the raw extract and centrifuge it for the phase separation between the aqueous phase and the n-hexane phase with 3000 x g. Dilute 7 mL of the aqueous phase with 43 mL PBS.

AflaCLEAN:

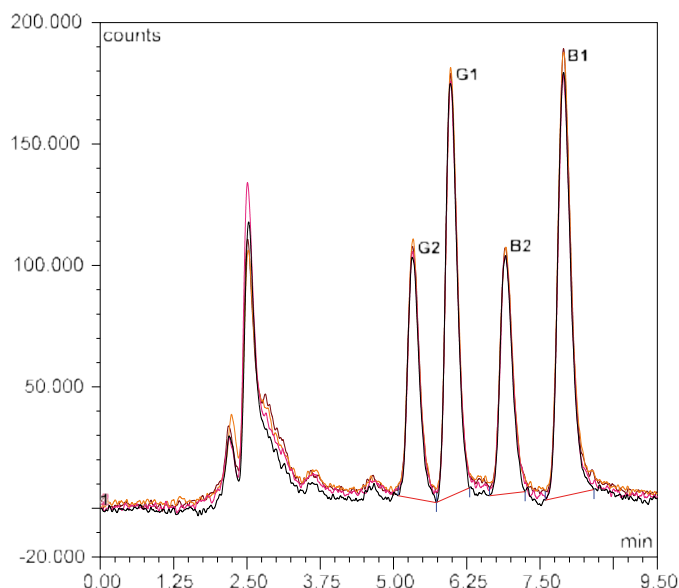
When using the immunoaffinity column AflaCLEAN, load the extract onto the AflaCLEAN column. Wash the column with deionised water and elute it with 2 mL of methanol. Keep in mind that the methanol incubates for 5 minutes into the column bed.

AflaCLEAN SMART:

For processing with the AflaCLEAN SMART column use only 10 mL of the diluted extract (represents 0.28 g matrix). Load the 10 mL onto the AflaCLEAN SMART column with a flow rate of 3 mL/min. Wash the column with 2 mL deionised water at the same flow rate. For elution, you only need 400 µL methanol and therefore have a comparable toxin concentration as with the 3 mL column.

➔ *With the AlfaCLEAN SMART column you save matrix, solvent and time.*

Chromatograms



Overlay of 4 chromatograms: 5 ppb peanuts cleaned-up with AflaCLEAN and AlfaCLEAN SMART columns

HPLC-Conditions

(Aflatoxin B/G)

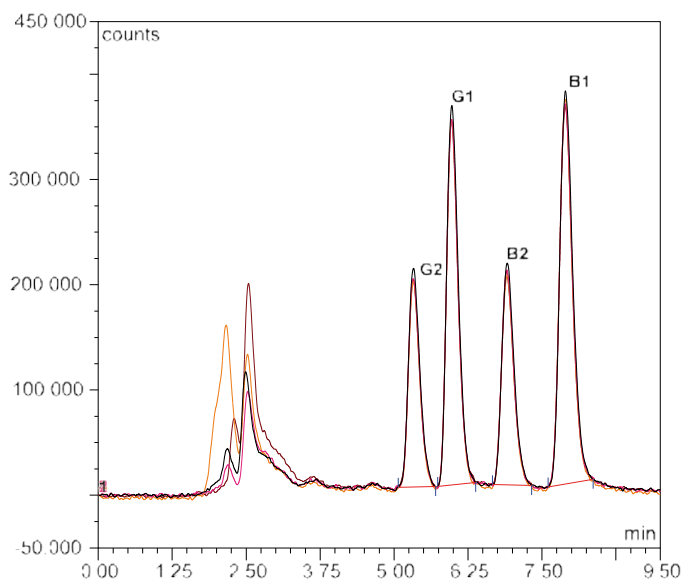
Mykotoxin	Aflatoxin B/G
HPLC:	isocratic
Column Oven:	36 °C
Separation Column:	RP C-18 (P/N 10544)
Flow Rate:	1.2 mL/min
Eluent:	HPLC-Water/Methanol/ Acetonitrile (60/30/15 (v/v/v))
Flourescence Detection:	Derivatisation with UVE Photochemical Reactor
Excitation Wavelength:	365 nm
Emission Wavelength:	460 nm

Recovery Rates

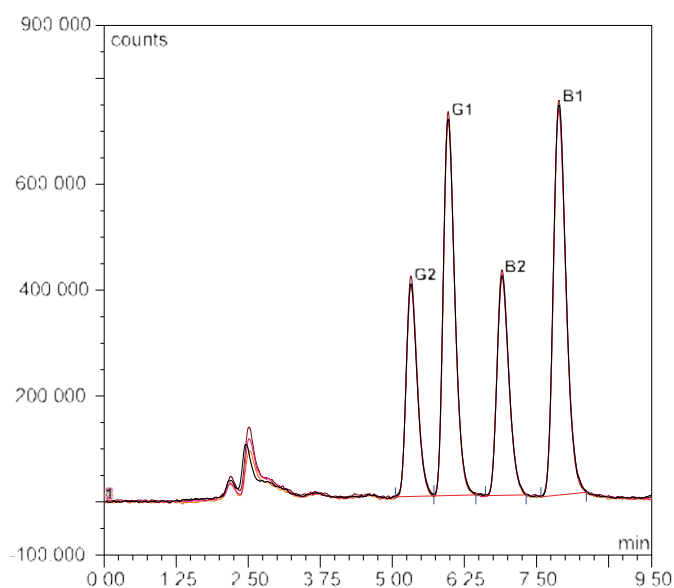
Content of Alfatoxin B/G in Peanuts

Aflatoxin B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Peanuts, 5 ppb	89	93	85	87
rel. error (%)	1	1	1	2
Recovery Rate** Peanuts, 10 ppb	92	91	86	87
rel. error (%)	1	1	2	2
Recovery Rate** Peanuts, 20 ppb	97	95	93	90
rel. error (%)	1	1	1	1

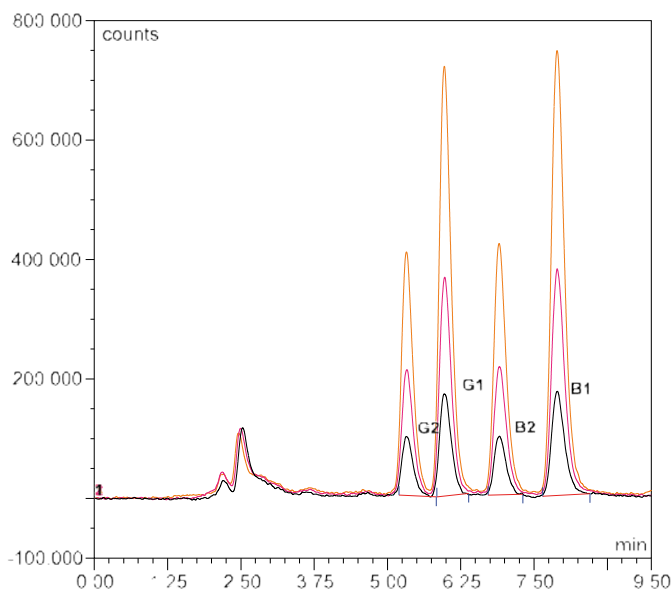
*Standard is set = 100 %, **Corrected with non-spiked sample /
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)



Overlay of 4 chromatograms: 10 ppb peanuts cleaned-up with AlfaCLEAN and AlfaCLEAN SMART columns



Overlay of 4 chromatograms: 20 ppb peanuts cleaned-up with AlfaCLEAN and AlfaCLEAN SMART columns



Overlay of 3 chromatograms:

Blck: 5 ppb peanuts cleaned-up with AlfaCLEAN SMART

Red: 10 ppb peanuts cleaned-up with AlfaCLEAN SMART

Orange: 20 ppb peanuts cleaned-up with AlfaCLEAN SMART



AlfaCLEAN SMART Column

Small + Fast + Economical = Clever & SMART

- Suitable for manual and automated processing, e.g. with the robotic system FREESTYLE ThermELUTE™
- Saving of up to 80 % of solvents
- Significant reduction in processing time for essential clean-up steps
- Very good recoveries for all matrices
- Reproducible results



These LCTech Products were used:

AlfaCLEAN Immunoaffinity Column for Aflatoxin B/G
P/N 10514 / 11721

AlfaCLEAN SMART Immunoaffinity Column for Aflatoxin B/G
P/N 12862 / 12863

HPLC Separation Column RP C-18
P/N 10522

FREESTYLE ThermELUTE™, Robotic System for Automated Sample Preparation
P/N 12663 / 12668 / 13691