



May 2019

Aflatoxin B/G in Almonds ~ 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

Almonds

At funfairs or Christmas market – the smell of roasted almonds enchants many people every year again. The popular almond is not a nut, but belongs to the genus of stone fruits. The origin of the almond is in the area from Asia Minor to Central Asia.

Almonds can be divided into two different types, sweet and bitter almonds. The bitter ones, which is however only toxic to humans in its raw state, contain a high proportion of hydrocyanic acid. In addition, almonds are among the top suppliers of vitamin E, beta-carotene, B vitamins and are also rich in fibers.

Whether the whole, grounded or rasped fruit – it can be used in many different variations. However, if stored incorrectly or during a manufacturing process, undesirable mould formation can occur, resulting in an excessively high aflatoxin B/G content. This is demonstrated by regular EU border controls, where excessively high aflatoxin B/G levels repeatedly lead to rejections in almonds. In 2018 only, 32 imports of almonds were rejected in their country of origin due to the high mycotoxin levels.

Automated Sample Clean-up: FREESTYLE SPE Makes it Possible

During day, night and even at weekend – the FREESTYLE system performs routine tasks in the field of mycotoxin analysis unattended around the clock, leaving you more time for other important tasks in the laboratory.

Each manual SPE method which has already proven in your laboratory can be transferred to the robotic system FREESTYLE SPE. Already created methods can be saved and recalled, but can also be modified.

The application fields are wide: from food and feed to environmental samples up to forensic applications and doping control samples.

Follow the instructions on the following page to perform the prepared processing steps. Then position the almond sample in the FREESTYLE SPE, change settings or parameters in the software with a few mouse clicks and start the system - done.



Robotic System FREESTYLE SPE

Processing Protocol

Homogenise 20 g almonds and add 2 g of sodium chloride. Extract the mixture through 100 mL 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 20 minutes.

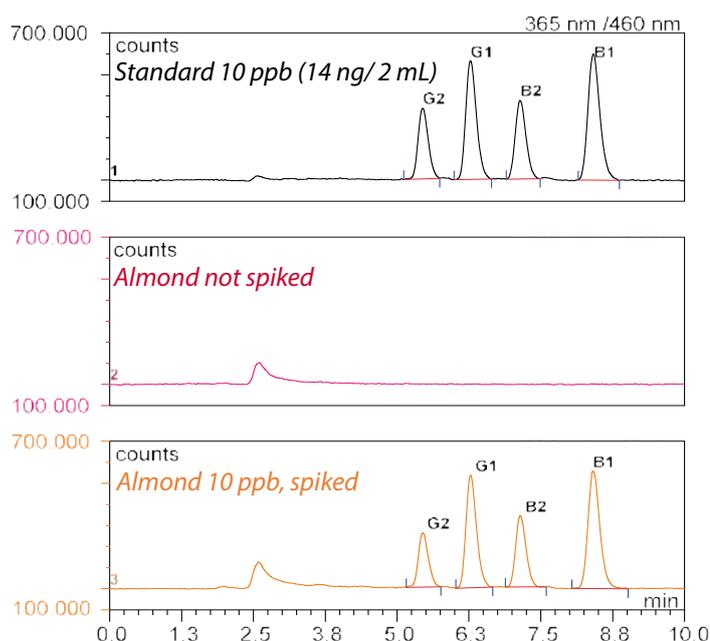
Filtrate the raw extract and dilute 10.5 mL of it with 64.5 mL PBS. In case of strong turbidity, filtrate the sample again through a glass fiber filter and load 50 mL (represents 0.4 g of matrix) of sample onto the AflaCLEAN immunoaffinity column with a maximum flow rate of 2 mL/min.

Wash the column afterwards with 2 x 5 mL deionised water. Dry the column with a short flush of air and elute the aflatoxins 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 toxin.

At the end dilute the eluate for HPLC-conditions and the sample can be analysed with fluorescence or LC-MS.

Chromatogram



HPLC-Conditions

(Aflatoxin B/G)

Mycotoxin:	Aflatoxin 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 Aflatoxin B/G in Almond

Aflatoxine B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Almond, 10 ppb	92	90	92	76

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

These LCTech Products were used:

AflaCLEAN
Immunoaffinity Columns for Aflatoxin B/G
P/N 10514 / 11721

HPLC Separation Column RP C-18
P/N 10522

FREESTYLE SPE, Robotic System
for Automated Sample Clean-up
P/N 12663 / 12668

UVE Photochemical Reactor
P/N 10519