



February 2018

Aflatoxins B/G in Dried Garlic

~ Manual and Automated with FREESTYLE SPE~

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 Marvellous Garlic

Did you know that garlic is also used in medicine? It keeps blood, heart and blood vessels healthy, disinfects the intestines and has remarkable pharmacological effects. Garlic contains the active ingredient „allicin“, which is responsible for the healing properties. Allicin fights harmful bacteria, viruses, larvae and fungi without any negative influence on the intestinal flora - unlike pharmaceutical antibiotics.

In order to ensure that the permissible pollutant regulations are observed when importing dried garlic, they must be constantly monitored for dangerous moulds (mycotoxins). This month we have examined garlic for you and checked for aflatoxins B/G. You'll find the manual processing protocol, a chromatogram and recovery rates on the next page.

Automated Sample Processing: FREESTYLE SPE Makes It Possible

The FREESTYLE system takes over daily routine tasks in the laboratory, but also offers users the unique opportunity to combine specific working steps that were previously carried out individually

And all this during day, at night and even at weekends.

Any manual method that has proven itself in your laboratory can be transferred directly to the system. New created methods can be saved and reused or modified afterwards.

Simply carry out the preparatory processing steps described on the following page. Position the sample in the FREESTYLE SPE afterwards, parameterize the method in the software with a few mouse clicks, and start the system - done.



Preparatory Processing Steps

Homogenise 10 g of dried garlic and add 2 g of sodium chloride. Extract the mixture through 100 mL methanol/water (80/20 (v/v)). In order to remove fat and essential oils, add 50 mL of n-hexane during initial extraction. Continue the extraction for at least 30 minutes to ensure high extraction efficiencies.

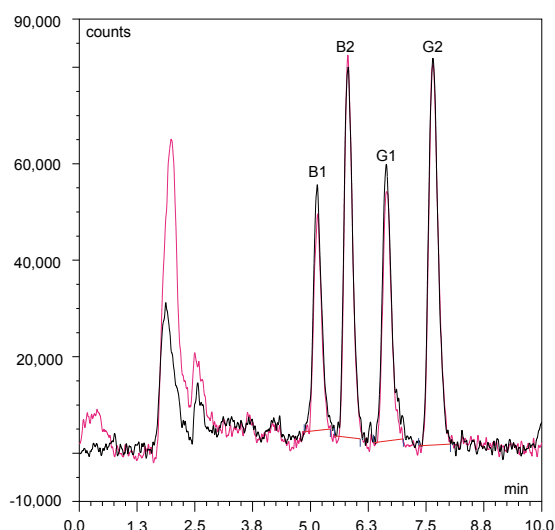
Protocol for Further Manual Processing

Filtrate the raw extract and dilute 2 mL of the n-hexane free (lower) phase with 12 mL of PBS (contains 8 % Tween). Afterwards, load 14 mL of sample (represents 0.2 g matrix) onto an immunoaffinity column AflaCLEAN Select.

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 of 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 toxins.



Chromatogram



Black: Standard 2 ng /2 mL

Red: Dried garlic spiked with 10 ppb before extraction

Immunoaffinity Columns for Aflatoxins B/G

The chromatogram shows the excellent results, which were obtained by using AflaCLEAN Select columns, with very high recovery rates and without any interferences.

For the analysis of Aflatoxins B/G LCTech offers different types of columns like for example AflaCLEAN and AflaCLEAN Select. Both are available in a convenient 3 mL polypropylene format. AflaCLEAN Select columns are also available in a 1 mL format. The columns are attractive for high sample throughput because of its markedly lower price, yet maintaining the same performance. Shelf life is 9 months cooled at 4 to 8 °C.

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:	Derivatization with UVE photochemical reactor
Excitation Wavelength:	365 nm
Emission Wavelength:	460 nm

Recovery Rates

Content of Aflatoxins B/G in Dried Garlic

Aflatoxins B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Dried Garlic, 10 ppb	98	92	95	84

*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 Select, Immunoaffinity Column
for Aflatoxins B/G
P/N 12062 / 12063

HPLC-Separation Column for Aflatoxins B/G
P/N 10522

UVE Photochemical Reactor
P/N 10519

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