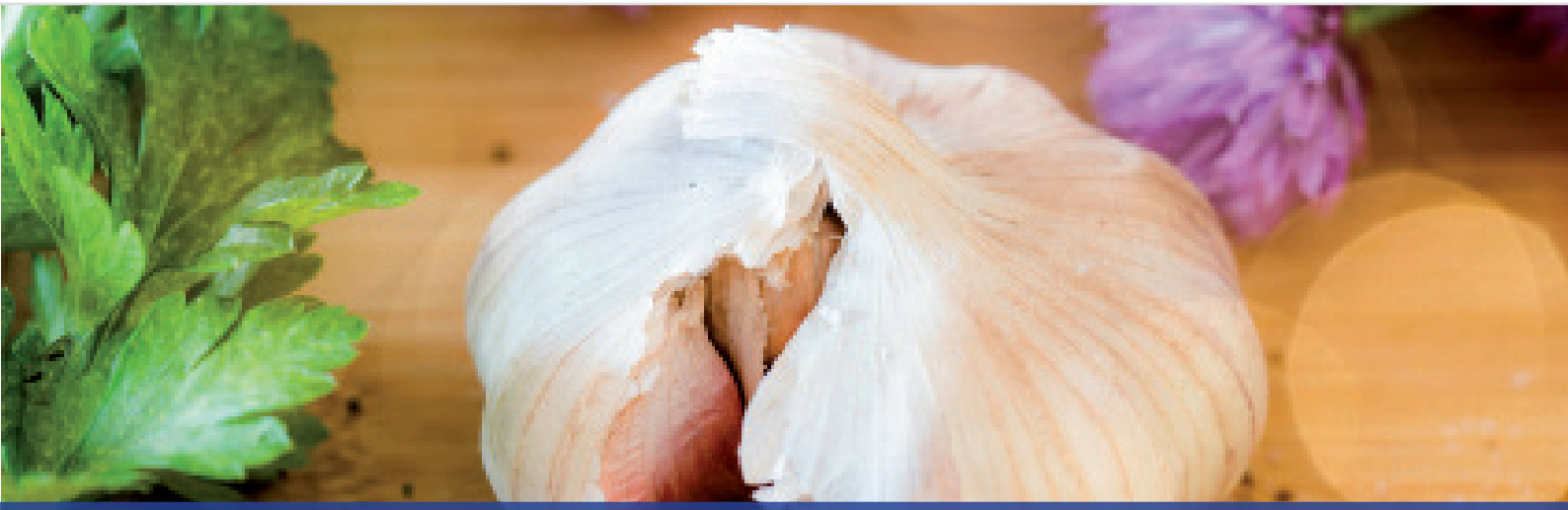
Matrix of the Month



July 2019

Aflatoxin B/G and Ochratoxin A in Garlic ~ 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

Garlic

Some love it, others can't even smell the intense odour. Garlic belongs to the leek plant genus and was already used in the Middle Ages. First as an active ingredient against the black plague, later it could be found in almost every pharmacy due to its antibacterial effect. It is generally said, that garlic keeps blood, heart and blood vessels healthy.

Allicin is the substance that is responsible for the antimicrobial effect. It is a sulphurous, essential oil that intercepts free radicals in the body and gives it its typical taste. The original distribution area of the plant extends from Central Asia to northeastern Iran.

China is by far the most important country for the cultivation and export of garlic. Around 80 % of garlic is grown in China. Garlic is often dried as a spice or processed in encapsulated form.

In this process or in case of wrong storage mycotoxins may occur, which can be toxic to humans if the contamination is too high. For this reason, there are EU-wide regulations that set the limit value for mycotoxins.

Automated Sample Preparation with FREESTYLE SPE

From the filtered and diluted raw extract to chromatogram without any manual intermediate steps. This is automated possible with FREESTYLE SPE. The robotic system works unattended 24/7 on your daily routine tasks in the field of mycotoxin analysis.

This saves time in the laboratory for other important tasks. The areas of applications ranges from food and feed to environmental samples, forensic applications and doping samples.

Any manual SPE method that has already proven in your laboratory can be transferred directly to the system. You can save, reuse and modify methods at any time.

Matrix of the Month

Processing Protocol for Aflatoxin B/G

Homogenise 10 g of garlic and add 2 g 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 essential oils.

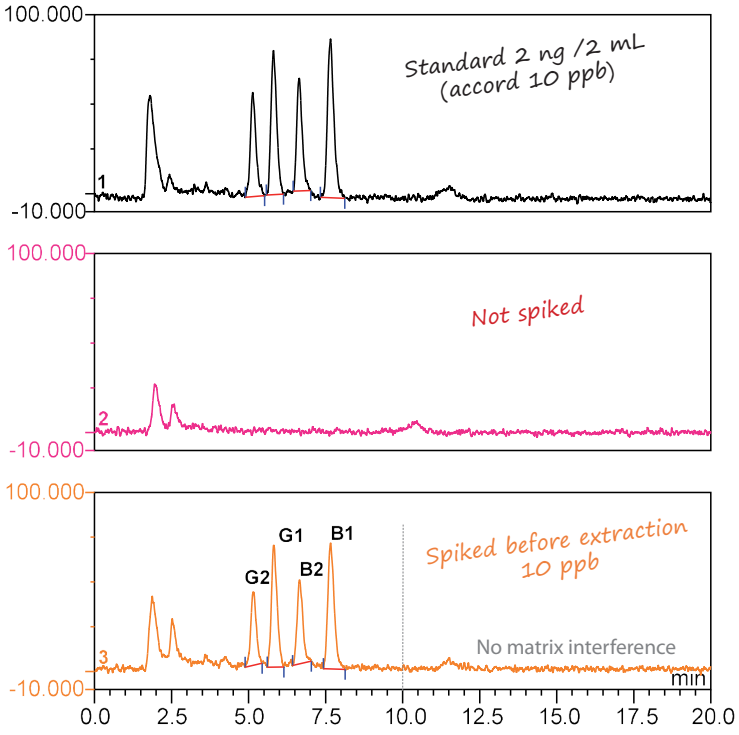
To ensure high extraction efficiencies, continue the extraction for at least 30 minutes.

Filtrate the raw extract and dilute 2 mL of the n-hexane free phase with 12 mL PBS (contains 8 % Tween). Load 14 mL of the sample onto an AflaCLEAN immunoaffinity column. Wash the column afterwards with 2 x 5 mL deionized water to remove efficiently the detergent residues. Elute the column 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.

Chromatogram



HPLC-Conditions
(Alfatoxin B/G)

Mycotoxin	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/Acetonitril (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 Aflatoxin B/G in Garlic

Mycotoxine	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Garlic, 10 ppb (AflaCLEAN)	93	87	98	91

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

Analysis of Aflatoxins and Ochratoxins

LCTech developed the immunoaffinity column Afla- and OtaCLEAN for automated sample preparation within routine analysis. The AflaCLEAN column is designed for clean-up of aflatoxins B1, B2, G1 and G2 in food and feed. On the other hand the OtaCLEAN column is able to bind ochratoxin with a very high specificity. Both columns also achieve very good recovery rates even in difficult matrices.

LCTech has developed a combined immunoaffinity column Alfa-OtaCLEAN for the analysis of both mycotoxins. A sample can be tested for ochratoxins and aflatoxins in one step. On the following page, you will find a protocol for testing of ochratoxin A in garlic.

Matrix of the Month

Processing Protocol for Ochratoxin A

Homgenise 20 g dried garlic and add 2 g of sodium chloride. Extract the mixture through 100 mL of methanol/water (80/20 (v/v)) and 50 mL n-hexane in order to remove fat and essential oils.

To ensure high extraction efficiencies, continue the extraction for at least 10 minutes. Filtrate the raw extract and dilute 2 mL of the sample with 12 mL PBS (contains 8 % Tween).

Load 14 mL sample onto an OtaCLEAN immunoaffinity column. Wash the column with 10 mL deionized water to remove efficiently the detergent residues. Give the wash solution in portions on the column.

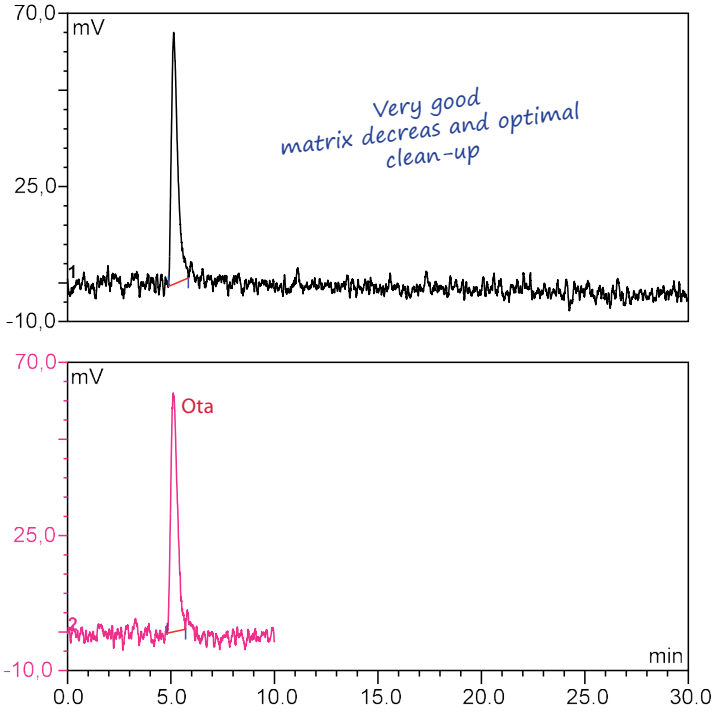
As above for the AflaCLEAN column, elute the column with 2 mL methanol. Keep in mind that the column bed is incubated with methanol for 5 minutes in order to ensure a fully denaturation of the antibodies and release of the toxin.

At the end, dilute the eluate for HPLC-conditions and afterwards inject the sample.

HPLC-Conditions
(Ochratoxin A)

Mycotoxin	**Ochratoxin A**
HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	RP EC 125/3 nucleosil 120-3 C18
Flow Rate:	0.6 mL/min
Eluent:	HPLC-Water/Methanol/Acetonitrile (40/55/5 (v/v/v)) + 1 % Acetic Acid
Flourescence Detection:	Without Derivatisation
Excitation Wavelength:	335 nm
mission Wavelength:	465 nm

Chromatogram



Black: *Garlic 10 ppb, cleaned-up with OtaCLEAN*
Red: *Standard 10 ppb (4 ng/ 2 mL)*

Recovery Rates
Content of Ochratoxin A in Garlic

Mykotoxine	OTA
Standard*	100
Recovery Rate** Garlic, 10 ppb (OtaCLEAN)	96

*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:

OtaCLEAN Immunoaffinity Columns for Ochratoxin A
P/N 10515 / 11535

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 Preparation
P/N 12663 / 12668

UVE Photochemical Reactor
P/N 10519

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