Matrix des Monats

November 2019

Aflatoxin B/G and Ochratoxin A in Chocolate ~ 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: info@LCTech.de

Sample Preparation

MYCOTOXINS

Chocolate

Chocolate has become an indispensable part of our daily life. Hardly any Central European can do without chocolate for a month. Whether as a spread on bread, as a biscuit filling, in cakes or simply as a small „thank you" - everyone loves it. The sweet pleasure stimulates our body to release the happiness hormone serotonin. This works directly in the brain and we feel balanced, satisfied and happy.

The cocoa beans needed to manufacture the chocolate are mainly imported from the Ivory Coast and other countries around the equator. As the beans have a high water content after harvesting, they must be dried in their country of origin before being imported into Germany or further processed.
Mycotoxins can be produced in the cocoa bean during the drying process or under incorrect storage conditions. These can be toxic at high concentration.

Sample Clean-up Made Easy by Automation with FREESTYLE SPE

During day, night and even at weekend - the automated FREESTYLE system takes over your daily routine tasks in the field of mycotoxin analysis unattended around the clock so that you have more time for other important tasks in the laboratory.

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

The fields of application range from food and feed samples to environmental samples, forensic applications and doping samples.

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

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Processing Protocol

Homogenise 10 g of chocolate and add 2 g of sodium chloride to the sample. Extract the mixture through 100 mL of methanol/water (80/20(v/v)) and add 50 mL n-hexane 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 it with 12 mL PBS (contains 8 % Tween). In the next step, load 14 mL of the sample onto an Afla-OtaCLEAN immunoaffinity column.

To enable efficient binding of the toxins to the antibodies, the flow rate should not exceed 2 mL / min.

Rinse the column with 10 mL deionized water and also load the rinse solution onto the column to remove matrix interferences from the column.

Dry the column with a short air flow and elute the toxin with 2 mL of methanol. Make sure that the column bed incubates with methanol for 5 minutes in order to ensure a fully denaturation of the antibodies and release of the toxin.

HPLC-Conditions
(Alfatoxin B/G / Ochratoxin A)

Mycotoxin	Aflatoxin B/G	Ochratoxin A
HPLC:	isocratic	isocratic
Column Oven:	36 °C	40 °C
Separation Column:	RP C-18 (P/N 10522)	RP EC 125/3 nucleosil 120-3 C18
Flow Rate:	1.2 mL/min	0.6 mL/min
Eluent:	HPLC-water/ methanol/acetonitrile (60/30/15 (v/v/v))	HPLC-water/ methanol/acetonitrile (40/55/5 (v/v/v)) +1% Acetic Acid)
Fluorescence Detection:	Derivatisation with UVE Photochemical Reactor	without Derivatisation
Excitation Wavelength:	365 nm	335 nm
Emission Wavelength:	460 nm	465 nm

Recovery Rates
Content of Alfatoxin B/G in Chocolate

Aflatoxin B/G	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate Milk Chocolate, 10 ppb**	98	98	84	92
Recovery Rate Noisette Chocolate, 10 ppb**	102	99	86	95
Recovery Rate Dark Chocolate, 10 ppb**	96	91	100	98
Recovery Rate Grape-Nut Chocolate, 10 ppb**	102	93	90	82
Recovery Rate Rum-Grape-Nut-Chocolate, 10 ppb**	100	100	90	94

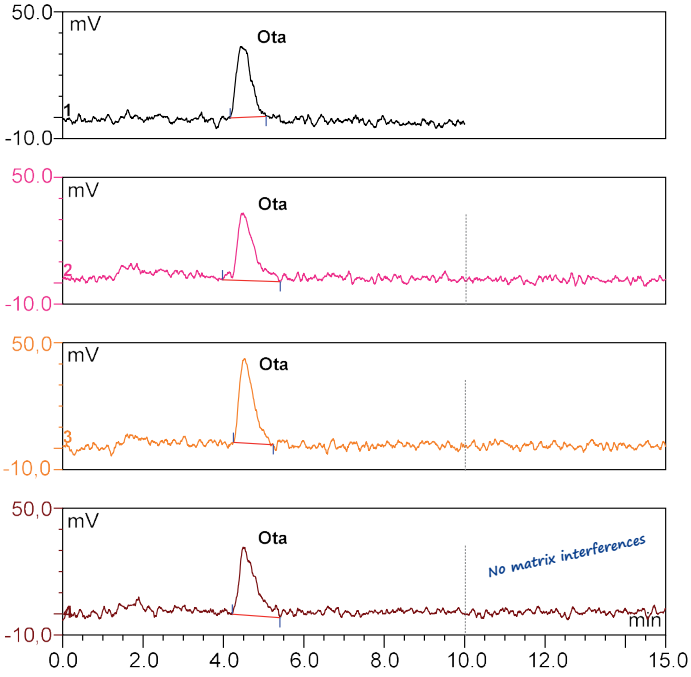
*Standard is set = 100%, **Corrected with non-spiked sample /
The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

Recovery Rates
Content of Ochratoxin A in Chocolate

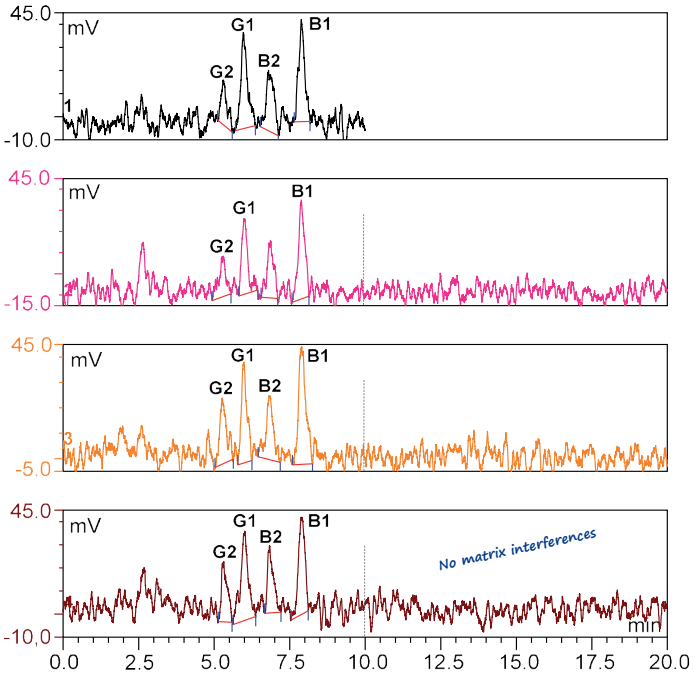
Mycotoxin	Ochratoxin A
Standard*	100
Recovery Rate Milk Chocolate, 5 ppb**	95
Recovery Rate Noisette Chocolate, 5 ppb**	99
Recovery Rate Dark Chocolate, 5 ppb**	99
Recovery Rate Grape-Nut Chocolate, 5 ppb**	94
Recovery Rate Rum-Grape-Nut-Chocolate, 5 ppb**	94

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

Matrix of the Month



Black = Standard 5 ppb (2 ng/2 mL)
Red= Milk Chocolate, cleaned-up 5 ppb
Orange = Noisette Chocolate, cleaned-up 5 ppb
Brown = Dark Chocolate, cleaned-up 5 ppb



Black = Standard 10 ppb total Aflatoxin (B1/G1 represents 4 ppb; B2/G2 represents 1 ppb)
Red = Milk Chocolate, cleaned-up 10 ppb
Orange = Noisette Chocolate, cleaned-up10 ppb
Brown = Dark Chocolate, cleaned-up 10 ppb

All in one – Fast and Efficient

Aflatoxins B/G and Ochratoxin A are naturally occurring mycotoxins and are formed by fungi when stored moist or incorrectly. LCTech has developed the combined Afla-OtaCLEAN immunoaffinity column for the clean-up of aflatoxin B1, B2, G1, G2 and ochratoxin A to facilitate the extraction of the two mycotoxins. Since LCTech produces both antibodies and clean-up columns, extensive quality tests during the entire production process ensure high product quality.

Conclusion

The recovery rates show an efficient enrichment of the toxins even for difficult matrices, such as cocoa-containing desserts. Chromatographically, no noticeable intereferences can be detected within 10 - 15 minutes. This enable a fast and clean analysis of your samples within 10 minutes.

Immunoaffinity Column Afla-OtaCLEAN, for Aflatoxins B/G and Ochratoxin A

These LCTech Products were used:

Afla-OtaCLEAN Immunoaffinity Column for Aflatoxin B/G and Ochratoxin A
P/N 11022 / 11771

HPLC Separation Column RP C-18
P/N 10522

UVE Photochemical Reactor
P/N 10519

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

LCTech GmbH, Daimlerstr. 4, 84419 Obertaufkirchen, Germany, Tel. +49 8082 2717-0, Fax +49 8082 2717-100, info@LCTech.de, www.LCTech.de