Matrix of the Month

LCTech wishes you Merry Christmas!

December 2018

Aflatoxin B/G and Ochratoxin A in Ginger and Aniseed ~ 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

Spices of The Christmas Season

The scent of Christmas spreads everywhere. The Christmas markets are open and tempt with mulled wine, cookies and other delicacies. Ginger, cinnamon, aniseed and cloves are among the most popular Christmas spices.

However, spices are often susceptible to the occurence of mycotoxins such as aflatoxin B/G and ochratoxin A, especially when improperly processed or stored. For this reason, the maximum levels of mycotoxins are strictly regulated by law. However, dyestuffs, essential oils or other matrix interferences contained in the spices often make an investigation rather challenging.

Clean-up of Aflatoxins B/G and Ochratoxin A

Aflatoxins and ochratoxin A are often found together in spices. To facilitate the work and to halve the working time, LCTech has developed the combined immunoaffinity column Afla-OtaCLAN for the clean-up of aflatoxin B1, B2, G1, G2 and ochratoxin A simultanously in comparison to the AflaCLEAN and OtaCLEAN columns.

The columns are available in the convenient 3 mL polypropylene format, they can be processed automatically (e.g. with the LCTech robotic system FREESTYLE SPE) and can be stored at room temperature for 18 months from the date of manufacture.

The chromatographic results are excellent, without interfering signals and with high recoveries, even for difficult matrices such as spices. Convince yourself on the following pages. We have taken a closer look at ginger and aniseed using the immunoaffinity columns AlfaCLEAN, OtaCLEAN and Afla-OtaCLEAN to compare the column clean-up performance and recoveries of aflatoxin B/G and ochratoxin A.

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Processing Protocol

Homogenise 10 g of matrix (ginger or aniseed) and add 2 g of sodium chloride. Extract the sample through 100 mL of methanol/water (80/20 (v/v)) and add 25 mL n-hexane in order to remove fat and essential oils. For high extraction efficiencies, continue the extraction for 30 minutes.

Filtrate the raw extract and dilute 2 mL of it with 12 mL of PBS (contains 8 % Tween20). Load 14 mL of the sample (represents 0.2 g of matrix) onto the respective immunoaffinity column Afla-OtaCLEAN, AflaCLEAN or OtaCLEAN. Wash the column with 10 mL deionised water each and dry it afterwards by flushing air through it.

Elute the toxin with 2 mL of methanol. Keep in mind that the column bed is incubated with methanol for 5 minutes to ensure a fully denaturation of the antibodies and release of toxins.

Dilute the sample to eluent conditions and measure it afterwards via HPLC with fluorescence detection or LC-MS.

HPLC-Conditions
(Aflatoxin 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 C-18 (P/N 10522)
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
Flourescence Detection:	Derivatisation with UVE Photochemical Reactor	without Derivatisation
Excitation Wavelength:	365 nm	335 nm
Emission Wavelength:	460 nm	465 nm

Recovery Rates
Content of Ochratoxin A in Ginger and Aniseed

Mycotoxin	Ochratoxin A
Standard*	100
Recovery Rate** Ginger, 20 ppb (Afla-OtaCLEAN)	95
Recovery Rate** Ginger, 20 ppb (OtaCLEAN)	95
Recovery Rate** Aniseed 20 ppb (Afla-OtaCLEAN)	91
Recovery Rate** Aniseed, 20 ppb (OtaCLEAN)	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)

Recovery Rates
Content of Aflatoxin B/G in Ginger and Aniseed

Aflatoxin	B1	B2	G1	G2
Standard*	100	100	100	100
Recovery Rate** Ginger, 20 ppb (Afla-OtaCLEAN)	107	107	92	100
Recovery Rate** Ginger, 20 ppb (OtaCLEAN)	104	88	89	93
Recovery Rate** Aniseed 20 ppb (Afla-OtaCLEAN)	92	98	90	95
Recovery Rate** Aniseed, 20 ppb (OtaCLEAN)	89	84	94	89

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

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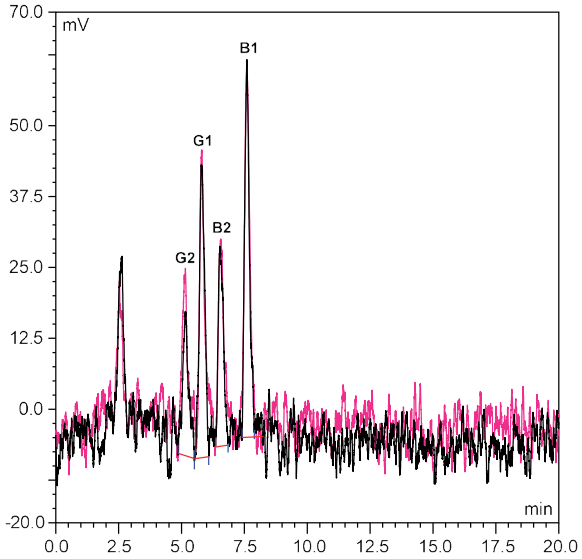
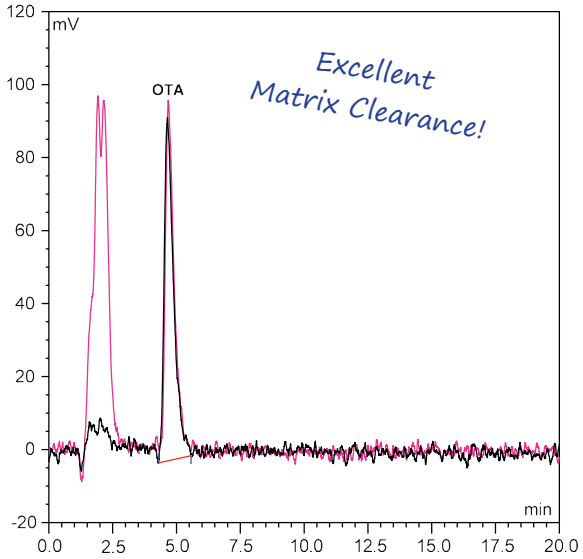
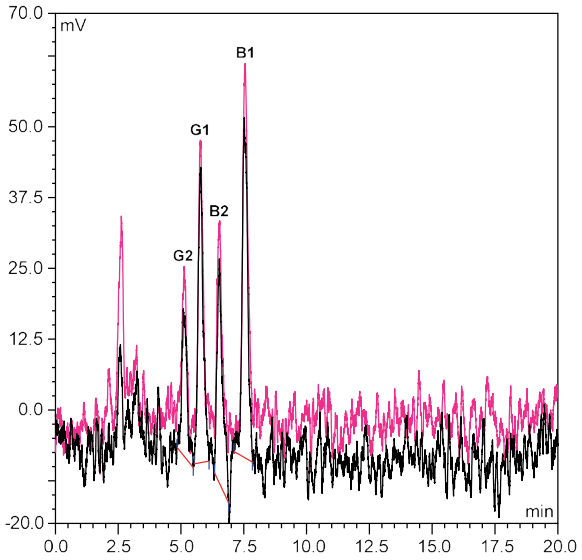
Chromatograms



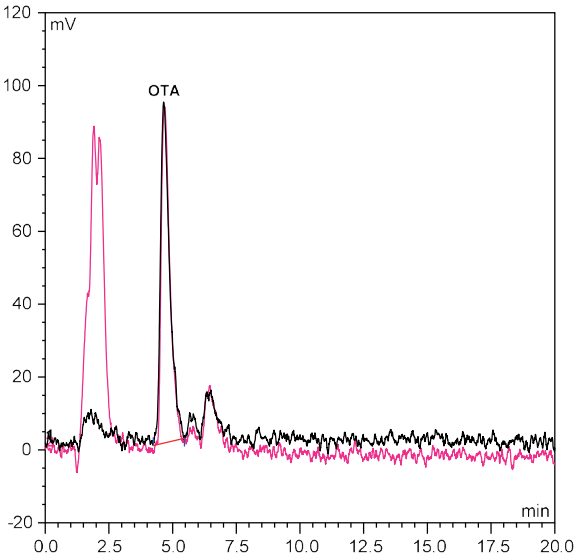
Red: 20 ppb Ginger cleaned-up with Afla-OtaCLEAN
Black: 20 ppb Ginger cleaned-up with AflaCLEAN



Red: 20 ppb Ginger cleaned-up with-OtaCLEAN
Black: 20 ppb Ginger cleaned-up with OtaCLEAN



Red: 20 ppb Aniseed cleaned-up with Afla-OtaCLEAN
Black: 20 ppb Aniseed cleaned-up with AflaCLEAN



Red: 20 ppb Aniseed cleaned-up with Afla-OtaCLEAN
Black: 20 ppb Aniseed cleaned-up with OtaCLEAN

Conclusion

Even in highly contaminated batches, the Afla-OtaCLEAN immunoaffinity columns allow the combined analysis of both toxin groups, saving not only time but also money.

The overlays of the chromatograms show that comparably good results are achieved with the single columns AflaCLEAN and OtaCLEAN as well as with the combined Afla-OtaCLEAN.

No interference peaks can be seen within the 20 minutes as shown here. The chromatography time can thus be reduced to outstanding 10 minutes, which increases the sample throughput.

These LCTech Products were used:

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

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

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

HPLC Separation Column RP C-18
P/N 10522

UVE Photochemical Reactor
P/N 10519

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