

Matrix of the Month

August 2015:

**Aflatoxins B/G and
Ochratoxin A in Raisins:
Fully Automated with
FREESTYLE ThermELUTE™**



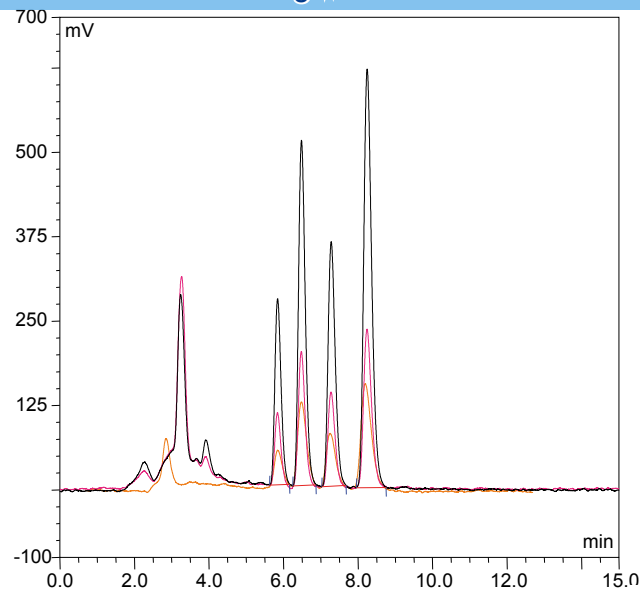
Do you have a special matrix that we should test for mycotoxins
Please let us know and write an e-mail to info@LCTech.de!

Fully Automated Sample Preparation and Analysis

Automate the sample preparation in your laboratory and let the robotic system FREESTYLE ThermELUTE™ handle your samples from raw extract to chromatogram:
24 hours / day for 7 days / week.

Due to the unique technology of thermal denaturation combined with the miniaturisation of the process you need less sample. At the same time you have less solvent consumption and achieve more sensitive and reproducible results in the lower ppt-range and a higher throughput.

Automated Processing with FREESTYLE ThermELUTE™



Raisins, 10 ppb aflatoxins

Black line:
FREESTYLE ThermELUTE™, 2.5 mL sample

Red line:
FREESTYLE ThermELUTE™, 1 mL sample

Orange line:
Manual processing with AflaCLEAN column;
50 mL sample

Using the FREESTYLE ThermELUTE™ the results become more sensitive thus even in ranges with very low limits, e.g. for baby food, the analytes can be reliably measured.

Due to the miniaturisation of the process using the SMART immunoaffinity columns AflaCLEAN SMART and OtaCLEAN SMART less sample, less solvent and less processing time is required.



Manual Processing →

Protocol of Processing

Extract 20 g of homogenised raisins and 2 g NaCl with 100 mL of the extraction solution (methanol/water, 80/20, v/v) and 50 mL n-hexane for at least 10 minutes for defatting. Filtrate the raw extract.

You can centrifuge it for 10 minutes at 2000 x g facilitating the phase separation.

Aflatoxin: Dilute 7 mL of the filtrate with 43 mL PBS. In case of precipitation or turbidity you can remove them by filtration. Apply max. 10 mL thereof onto the immunoaffinity column AflaCLEAN SMART by FREESTYLE ThermELUTE™.

Ochratoxin: Dilute 10 mL of the filtrate with 40 mL PBS. In case of precipitation or turbidity you can remove them by filtration. Apply max. 10 mL thereof onto the immunoaffinity column OtaCLEAN SMART by FREESTYLE ThermELUTE™. (Shown in the subsequent chromatogram: 3 mL of the filtrate are diluted with 12 mL PBS and 10 mL thereof are applied onto the immunoaffinity column OtaCLEAN SMART by FREESTYLE ThermELUTE™. This represents 0.08 g matrix.

The respective column is automatically loaded, washed with 2 mL water (Aflatoxin: 3 mL/min., Ochratoxin: 1.5 mL/min) and heated by the FREESTYLE system. Afterwards the eluate is quantitatively injected into the sample loop and analysed via HPLC-FLD.

HPLC Conditions

	Aflatoxins B/G	Ochratoxin A
HPLC:	Isocratic	Isocratic
Column oven:	36 °C	40 °C
Separation column:	RP C18	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:	Photochemical derivatisation with UVE	Without derivatisation
Excitation wavelength:	365 nm	335 nm
Emission wavelength:	460 nm	465 nm

Recovery Rates

Content of Aflatoxins B1, B2, G1, G2 and OTA in Raisins					
	Aflatoxin				Ochratoxin A
	B1	B2	G1	G2	OTA
Standard*	100	100	100	100	100
Recovery rate** Raisins 10 ppb	94	96	94	91	90

* Standard is set = 100 % , ** corrected with non-spiked sample



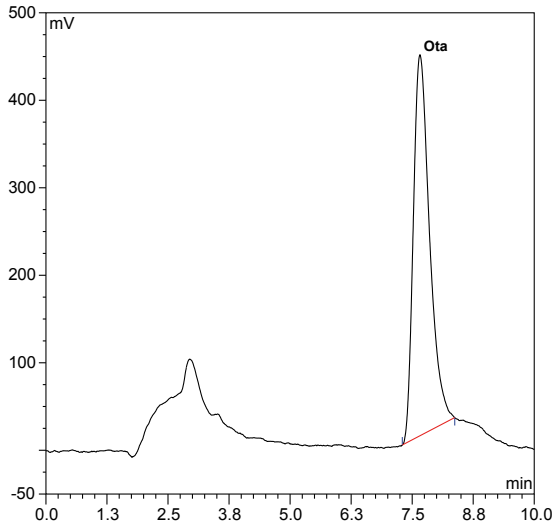
SMART columns with tip

Chromatograms →

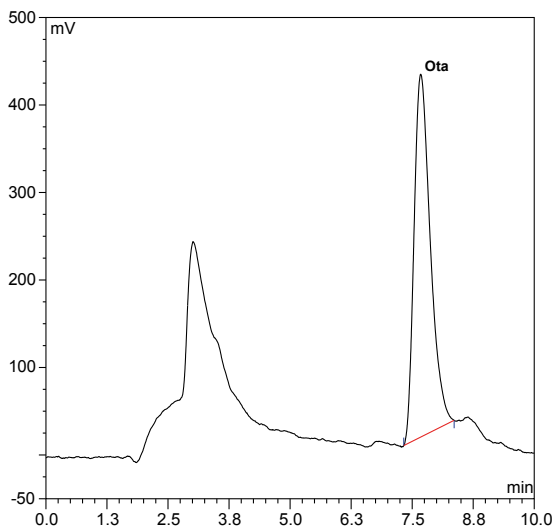
Chromatograms



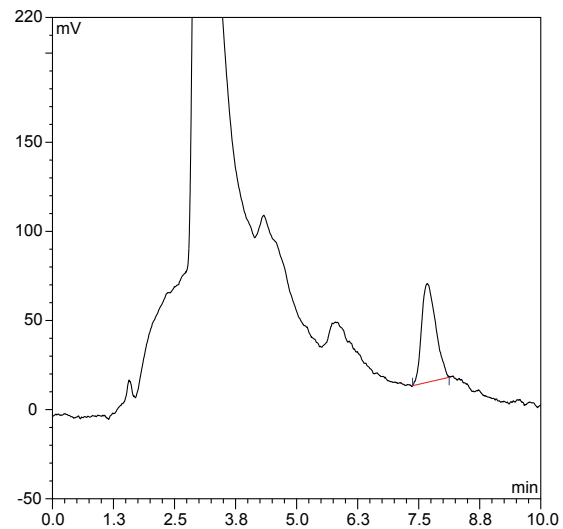
Fully automated, sensitive,
SMART



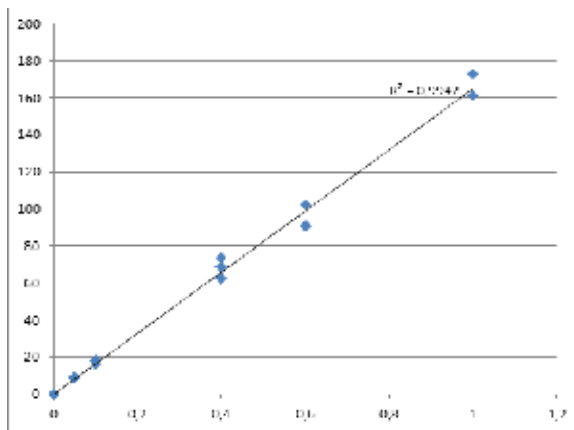
Ota standard, 10 ppb (0.8 ng / injection)



Raisins, 10 ppb (0.08 g loaded, spiked with 10 ppb OTA)



Raisins, 1 ppb OTA (0.08 g matrix were injected)



Calibration curve OTA (n=3):
(0-1.2 ng/10 mL represents 0-12.5 ppb);
LOQ 0.03 ppb (signal/noise 5:1)

These LCTech products were used:

AffaCLEAN SMART immunnoaffinity column for aflatoxins B1, B2, G1 and G2
P/N 12862 / 12863

OtaCLEAN SMART immunnoaffinity column for ochratoxin A
P/N 13346 / 13351

FREESTYLE ThermELUTE™ Robotic system for sample preparation and analysis
P/N 12663 / 12668 / 13691

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Do you have further questions?
Please just write an e-mail to info@LCTech.de!