LC Tech

October 2015:

Ochratoxin A in Pumpkin Seed

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 Mycotoxin Analysis

Fully automated mycotoxin analysis from raw extract to chromatogram - quite simple with the robotic system FREESTYLE ThermELUTE™. The samples are automatically loaded, washed, thermally eluted and quantitatively transferred into the injection system of any LC system via partial filling. Due to the injection of the complete eluate and the omission of the adjustment of the HPLC eluent extreme low detection limits in the lower ppt range can be reached.
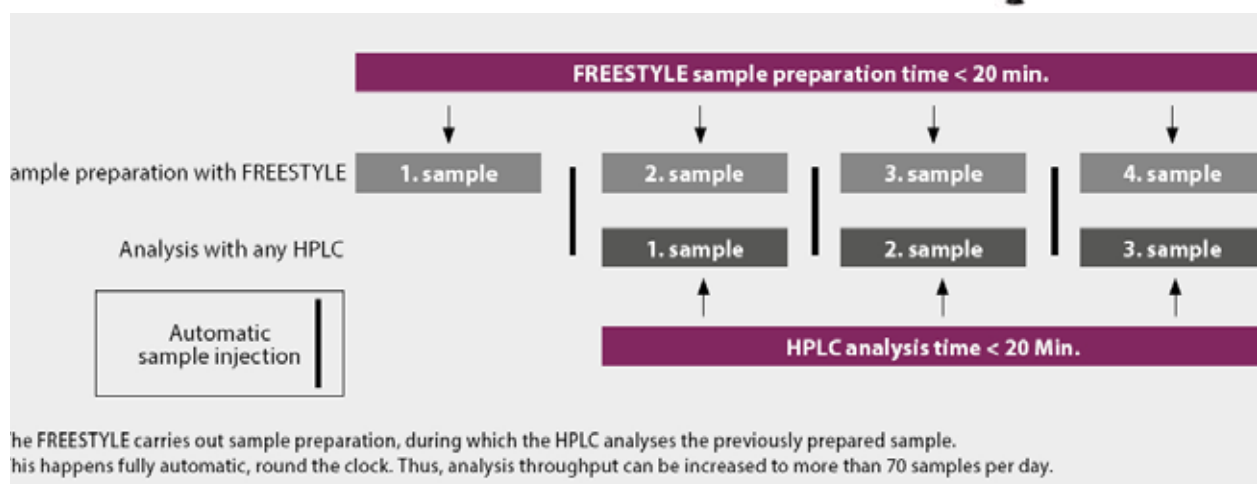
At the same time you increase your sample throughput up to 500 samples per week due to the parallel sample processing in both the robotic system and also in the LC system.

FREESTYLE ThermELUTE™

✓ Excellent results

✓ Best reproducibility

✓ No cross-contamination

ppt instead of ppb

FREESTYLE sample preparation time < 20 min.

| ample preparation with FREESTYLE | 1. sample | 2. sample | 3. sample | 4. sample |
| Analysis with any HPLC | 1. sample | 2. sample | 3. sample |

Automatic sample injection

HPLC analysis time < 20 Min.

he FREESTYLE carries out sample preparation, during which the HPLC analyses the previously prepared sample. his happens fully automatic, round the clock. Thus, analysis throughput can be increased to more than 70 samples per day.

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Manual Processing ➡

Protocol of Manual Processing

Extract 20 g of homogenised pumpkin seed 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. Dilute 10 mL of the filtrate with 40 mL PBS. In case of precipitation or turbidity you can efficiently remove them by filtration.

Now you have two choices. Either load the sample directly onto the immunoaffinity column OtaCLEAN SMART via FREESTYLE ThermELUTE™ resulting in a low range measurement. Alternatively dilute 3 mL of the filtrate again with additional 12 mL PBS and to load 10 mL (represents 0.08 g matrix) onto the column. Ideally the maximum matrix load is 0.4 g/injection.

The loading of the column (1.5 mL/min) is already done by the FREESTYLE system. The sample is then washed with 2 mL of water (1.5 mL/min) and thermally eluted. The eluate is quantitatively injected into the sample loop and analysed via HPLC-FLD.

HPLC Conditions

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
Fluorescence detection:	without derivatisation
Excitation wavelength:	335 nm
Emission wavelength:	465 nm

Recovery Rates

Content of Ochratoxin A in Pumpkin Seed	
Standard*	100
Recovery rate** Pumpkin seed, 10 ppb	102

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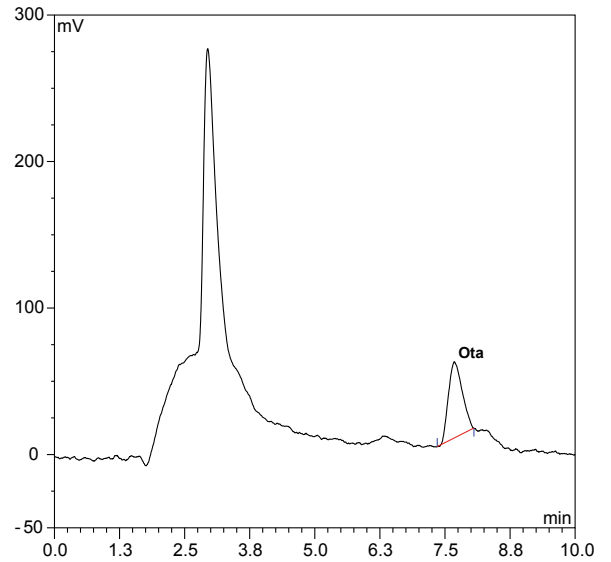
OtaCLEAN SMART with tip

Chromatograms →

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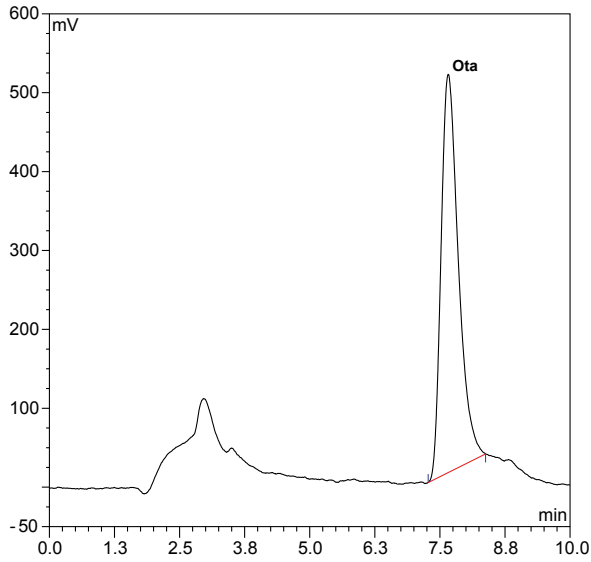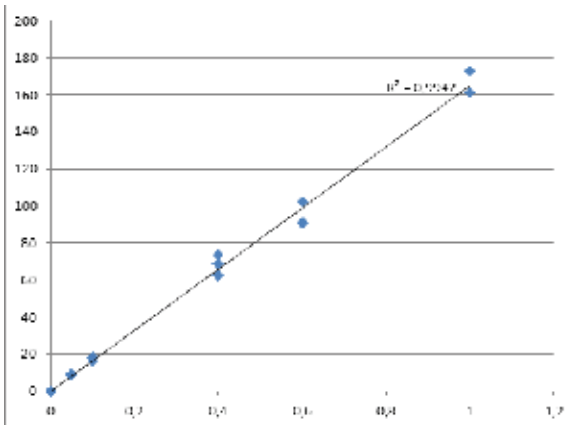
OTA standard, 0.8 ng/injection
(represents 10 ppb)
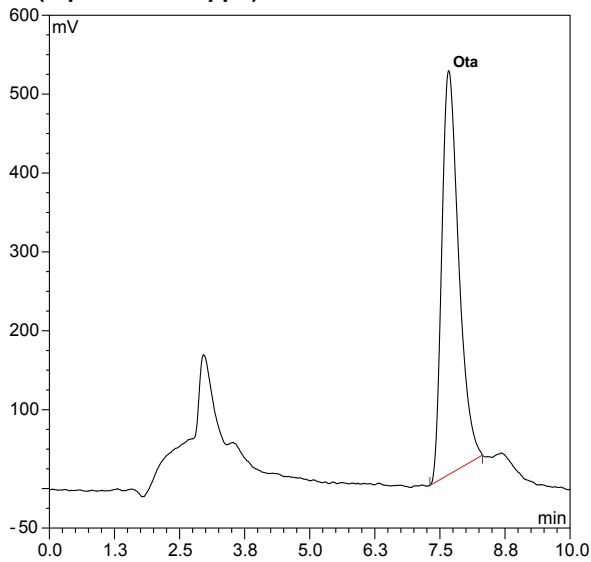
Pumpkin seed, spiked with 10 ppb OTA
(0.08 g matrix loaded)

Pumpkin seed, spiked with 1 ppb OTA
(injected 0.08 matrix)

Calibration curve (N=3)

Measurement range of 0-12.5 ppb

LOQ 0.03 ppb; (signal/noise 5:1)

depends on the detector sensitivity

These LCTech products were used:

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

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

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