

SOLUTIONS BY



# Determination of Ochratoxin A in Coffee via FREESTYLE ThermELUTE™ with Online HPLC-Measurement

## Determination of Ochratoxin A via FREESTYLE ThermELUTE™ with Online HPLC-Measurement

### Principle of the Method

Ochratoxin A is a highly toxic mycotoxin which is produced by various fungi during storage of matrices. The toxin (fig. 1) can be found in coffee, liquorice, spices, cereals and products made thereof. Due to the toxic effects on humans, the European Community (EC) has set maximum levels in several matrices in the Commission Regulation (EC) No 1881/2006 and amended by Commission Regulation (EU) 105/2010.

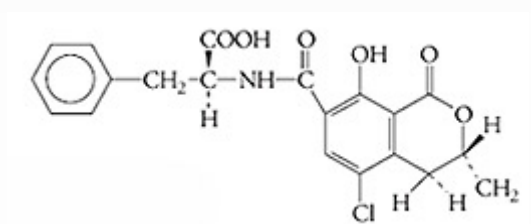


Fig. 1: Structure of Ochratoxin A

In this application note is described how the toxin, that was extracted according to the specifications, is automatically processed with the FREESTYLE ThermELUTE™ system, cleaned-up on the immunoaffinity column OtaCLEAN SMART, and injected online in the HPLC system with fluorescence detector for measurement.

Due to the special technology the time needed for the complete process including clean-up and measurement is only about 30 minutes. The analyte is thereby measured precisely in the lower ppt-range.

### Procedure

The samples of the respective matrix are extracted, diluted and filtrated according to the specifications of the OtaCLEAN SMART manual. An aliquot filled into a 16 mL vial is placed in the FREESTYLE ThermELUTE™ system. The immunoaffinity columns that are needed for the processing are put in a separate rack corresponding to the number of samples. Once the HPLC system is ready for use the automated processing can begin.

More than 70 samples per day can be completely handled and measured in a continuous process, because the subsequent sample is already handled by the FREESTYLE system while the previous sample is measured by the HPLC system.

## ThermELUTE™ and SMART Columns The Fast Combination

### Materials and Chemicals

1. FREESTYLE Basic P/N 12663
2. FREESTYLE SPE P/N 12668
3. FREESTYLE ThermELUTE™ P/N 13691
4. Adjustment tool for SMART columns P/N 13768
5. Special tray for 60 SMART columns P/N 13497
6. Frame, adjustable P/N 14062
7. Tray for 30 x 16 mL vials P/N 11933
8. Frame for trays P/N 11915
9. Screw-thread vials, 16 mL P/N V0016 (100 pcs/pck)
10. OtaCLEAN SMART P/N 13346 (100 pcs/pck) or  
P/N 13351 (1000 pcs/pck)
11. Injection needle P/N 13544 (1000 pcs/pck)
12. Methanol p.a.
13. Water p.a.
14. PBS buffer diluted 1 to 500
15. PBS buffer pH 7.4 containing 8% Tween20
16. Standard laboratory equipment including HPLC with fluorescence detector



Gripper takes adapter.



Adapter takes columns.



Column is transferred into the ThermELUTE™ module.

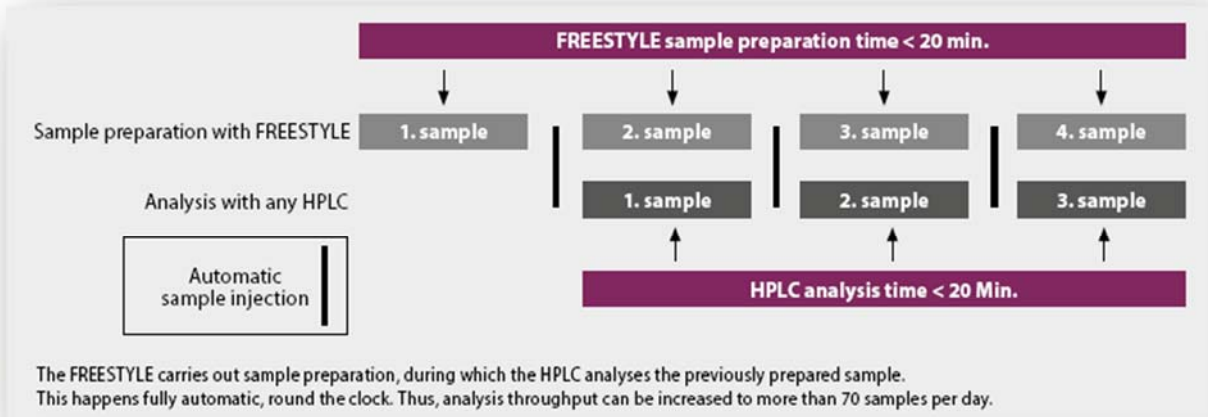
### Parameterization of the FREESTYLE ThermELUTE™ Method

# APPLICATION NOTE | AN0010

**FREE-STYLE™**  
AUTOMATISIERTE PROBEVORBEREITUNG

LCTech FreeStyle - Report on Methods: ThermElute Date: 14.01.2015 Time: 16:47:49

Name: OTA_2,8mL.tel		
Column:	OtaCLEAN_SMART.col	
Initial System Cleaning Port:	1	H2O
Sample Volume:	2.8 ml	Bottle type: Type1@16 ml
Flow rate sample:	1.5 ml / min.	
Port for 1. cleaning step after loading	7	40/60 MeOH/H2O
Port for 2. cleaning step after loading	1	H2O
Volume washing solution:	2 ml	
Flow rate washing solution:	1.5 ml / min.	
Port for 2. Washing solution:	9	1:500 PBS
Elution positioning volume:	810 µl Standard = 600 µl	
Flow rate eluting solution:	1.5 ml / min.	
Eluting solution:	8	Eluator
Temperature heating:	98 °C	
Heating time:	360 sec.	
Cooling time:	0 sec.	
Cleaning Step - Solvent from Port:	7	40/60 MeOH/H2O
Wait minimum process time for a longer HPLC measurement		5 min.
Use pressure limitation function during loading and washing		
Pressure limit for syringe pump		100 digits
If pressure limit is triggered:		
Time from start of sample to injection for HPLC		5 min.
Maximum count of triggered samples in series		3 Sample /s



## Results

### Recoveries and Chromatograms

#### Exemplary Recovery Rates

The matrices were processed according to the recommended extraction protocols and handled with the FREESTYLE ThermELUTE™.

Content of Ochratoxin A for various matrices:

Matrix	Matrix Load [g]	Recovery [%]
Chili	0.08	98
Coffee	0.08	98
Beer	1.428	97
Paprika	0.08	98
Rice	0.4	104

The samples were spiked with 5 ppb (5 µg/kg) respectively and the recovery rates were determined on the basis of a standard calibration curve.

The recovery rates were corrected by the quantity of toxin that was found in the samples that were not spiked.



## Exemplary Chromatograms

HPLC conditions:

Flow rate: 0.6 mL/min, acetonitrile / water / methanol (5/40/55 (v/v/v) containing 1% acetic acid)

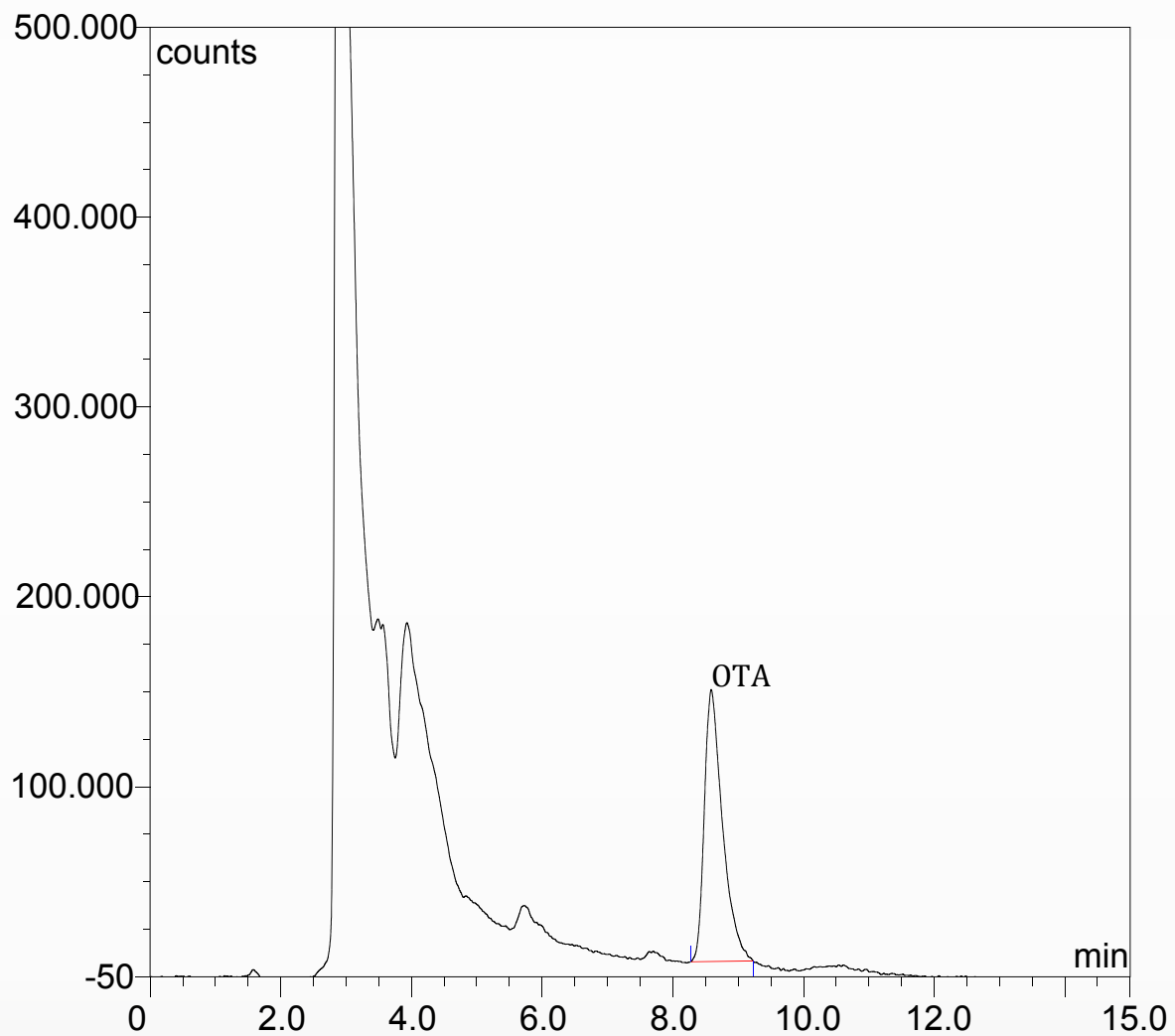
Separation column: RP C18 (LCTech P/N 10522)

Fluorescence detection: UltiMate™ 3000 RS fluorescence detector

Excitation wavelength: 335nm

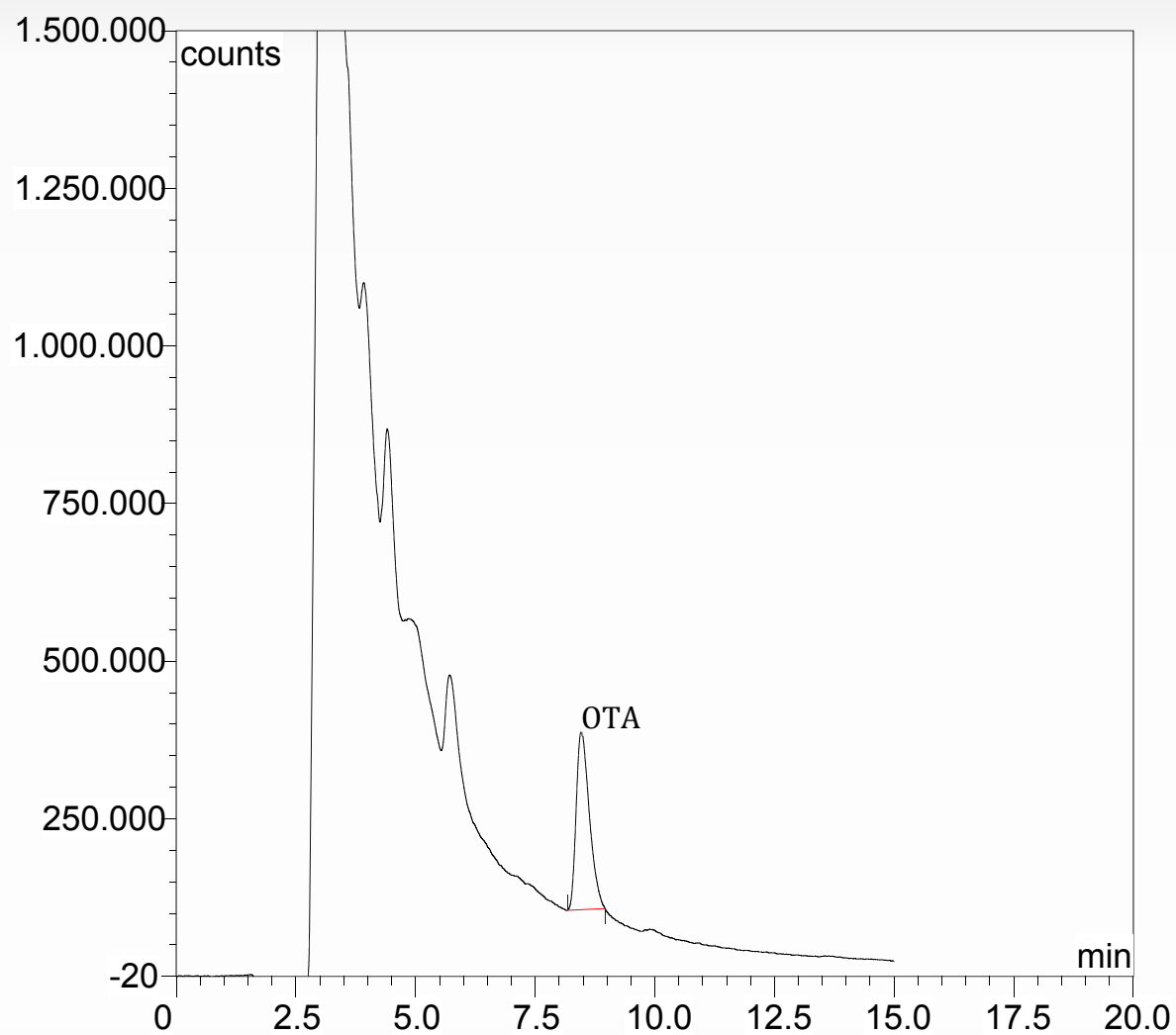
Emission wavelength: 465 nm

Chromatography of Ochratoxin A 0.1 ng in 2.8 mL sample (represents 1.25 µg/Kg (1.25 ppb)).

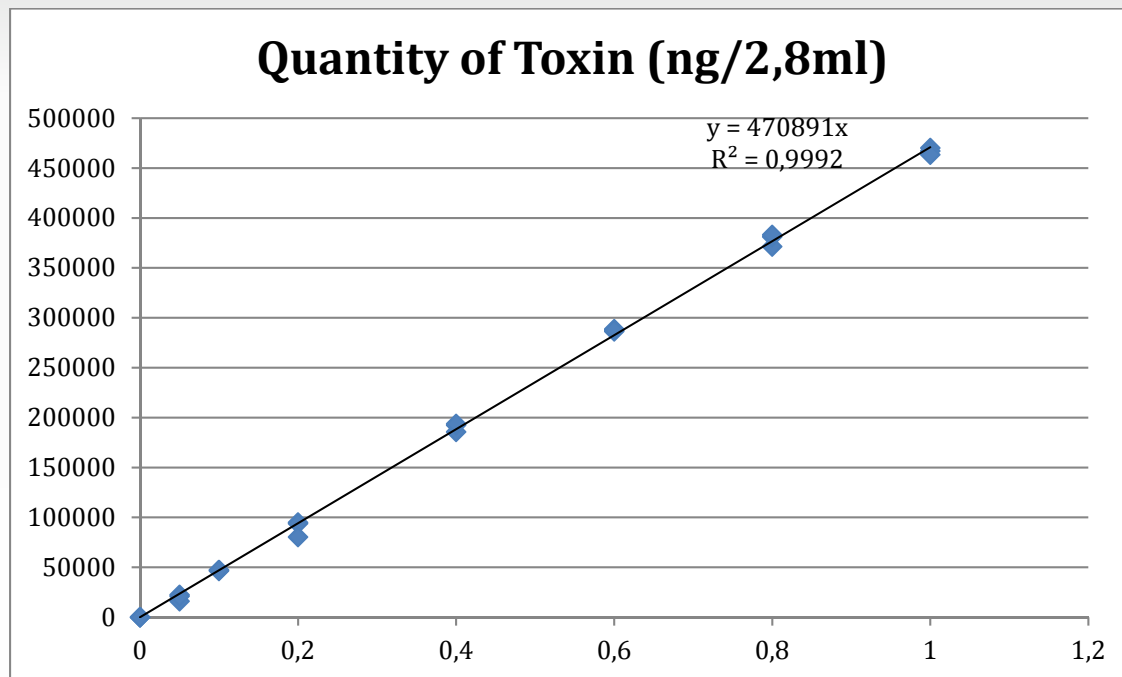


# APPLICATION NOTE | AN0010

Real contaminated coffee sample (no. 47594)



## Linearity and Reproducibility



From 0 to 1 ng / 2.8 mL (0 to 12.5 ppb) three standards were analysed and used for generation of the calibration curve. The correlation coefficient was calculated on all data points (8 (n=3)). The level of detection (LOD), which depends on the sensitivity of the fluorescence detector, was calculated 0.05 ppb (signal / noise ratio (1: 5)).

## Data Comparison

Comparison of the data that have been measured via FREESTYLE ThermELUTE™ using HPLC and fluorescence detection by LCTech and using HPLC and fluorescence detection by CR3 - Kaffeeveredlung M. Hermsen GmbH.

Sample	ThermELUTE™ ppb (µg/kg)	CR3 ppb (µg/kg)
1	0,38	0,36
2	0,77	0,77
3 (no. 47594)	3,34	4,4
4	0,15	< 0,2

Identical samples were extracted and analysed in the different laboratories. The extraction and the clean-up were performed according to the OtaCLEAN extraction and clean-up procedures. The data comparison shows a very good correlation.



## Literature and Regulations Acknowledgment

- COMMISSION REGULATION (EU) No 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A
- P. Good, H. Kandler, P. Koch und T. Schärer, *Mitt. Lebensm. Hyg.* 93, (2002) 186-193
- P. M. Scott and S. R. Kanhere, *Food Additives and Contaminants* 12 (4), (1995) 591-598
- Visconti, M. Pascale, G. Centonze, *J. Chrom. A* 864 (1999) 89-101
- Zimmerli, R. Dick, *J. Chrom. B Biomed. Appl.* 666 (1995) 85 – 99
- Thellmann, W. Weber, *DLR* 1 93 (1997) 1-3
- S. Kastrup, U. Aulwurm, *GIT* 9, (2002) 975-977

## Acknowledgment

We would like to express our sincere appreciation to Dr. Klaus Beckmann, Laboratory Manager at CR3 - Kaffeeveredlung M. Hermsen GmbH for providing the samples as well as the measured data.



**Contact**

LCTech GmbH  
Daimlerstraße 4  
84419 Obertaufkirchen  
Germany

Tel.: +49 8082 2717-0  
Fax: +49 8082 2717-100  
E-Mail: info@LCTech.de

[www.LCTech.de](http://www.LCTech.de)  
[www.LCTech-online.com](http://www.LCTech-online.com)

SOLUTIONS BY

