Sensitive Ochratoxin A Analysis
In Roasted Coffee

The adaptation of the limit values for ochratoxin A in coffee (to 3 ppb, (EU 2022/1370, 05.08.2022)) and also new levels for other matrices, which has been in force since 2022, accordingly leads to a necessary adaptation in the analytical measurement sensitivity. One way to achieve this sensitivity would be to implement LC/MS analysis, which in turn would reduce the capacity of these dedicated resources for more complex analytical measurements.

A great alternative to reach same sensitivity with HPLC-FLD is to set up the sample preparation with a ThermELUTE loaded with the corresponding minaturized SMART IAC columns to speed up the analysis. This comes along with a reliable full automation process from sample to chromatogramm, in accordance to regulations. Get to know this simple but efficient multiplying of the sensitivity by almost 14 times, compared to standard 3 mL IAC processing.

Product Highlights

Ochratoxin A analysis with high measurement sensitivity and low cost of analytical equipment, fast and precise with the OtaCLEAN™ smart column and the FREESTYLE ThermELUTE™:

- 24/7 sample clean-up
- Reproducible clean-up through parameterisable programmes
- Fast from crude extract to chromatogram in 20 minutes

- Highest measurement sensitivity
- Flexibility in the choice of application (aflatoxins / ochratoxin A / zearalenone)
- Suitable for all types of matrices (feed to baby food)

Processing Protocol

Mix 20 grams of coffee powder with 2 grams of sodium chloride, 100 mL methanol/water (80/20) and 50 mL n-hexanes and mix for 10 minutes. Filter the crude extract and centrifuge at 3000 x g, to optimise phase separation. The lower (n-hexane-free) phase is further used. Dilute 2 mL with 12 mL of PBS buffer containing 8 % Tween20 and transfer to a sample vial. Position the sample in the FREESTYLE and have the following steps processed fully automatically, with no user interaction. Load 2,8 mL onto an OtaCLEAN™ SMART column using the ThermELUTE™ process. The column is rinsed with 2 mL of aqueous wash solution and then, fully automatically by heat treatment, transferred the toxin in large volume into the ThermELUTE™ HPLC sample loop for chromatographic separation.

OtaCLEAN™ SMART

FREESTYLE ThermELUTE™

Do you have a special request as to which matrix we should test for you? Contact us by e-mail at: info@LCTech.de

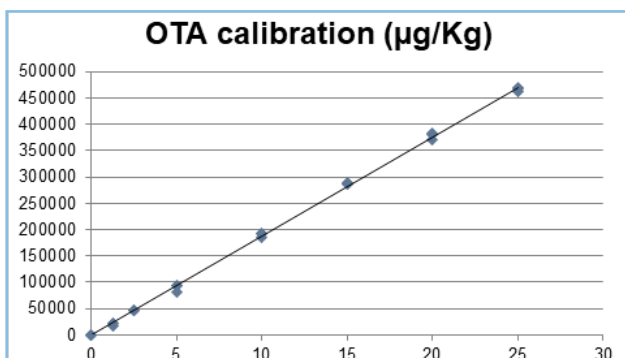## Parameter

Unit Parameters (FREESTYLE)	
Loading rate	1.5 mL/min
Loading volume	2.8 mL
Washing speed	1.5 mL/min
Wash volume	2 mL
Eluent	HPLC-water (98°C, 6 Minutes)
Elution Speed	1.5 mL/min
Elution volume	810 µL

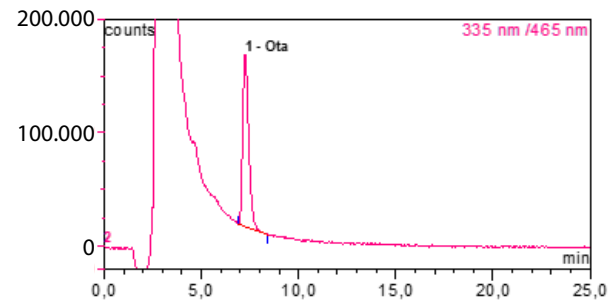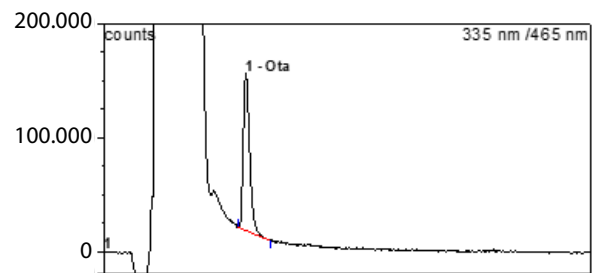| Chromatographic Parameters	
HPLC	isocratic
Column oven	40°C
Separation column	RP EC 125/3 nucleosil 120-3 C18
Flow rate	0.6 mL/min
Solvent	HPLC water/methanol/acetonitrile (40/55/5)+1% acetic acid
Fluorescence detection	without derivatisation
Excitation wavelength	335 nm
Emmission wavelength	465 nm

Coffee sample	OTA [ppb]	Reference values [ppb]
A	0,38	0,36
A	0,39	0,36
A	0,37	0,36
B	0,80	0,77
B	0,74	0,77
C	0,16	<0,2
C	0,13	<0,2

Roasted coffee (0.5 ppb), analysed with FREESTYLE ThermELUTE™ & OtaCLEAN™ SMART

Results

The linearity of the calibration by the OtaCLEAN™ SMART gives a high consistent correlation (0.9992). LOD / LOQ were calculated to be 0.048 ppb and 0.144 ppb for the coffee matrix, respectively. Additional calibration points were added to determine LOD and LOQ. The results of the coffee samples, whose content was not known when the analyses were carried out, correlate very well with those that could be determined in an official laboratory. The results of the analyses were available within 20 minutes of the start of the sample after completion of the calibration. This allows a fast reproducible and sensitive analysis over a very wide range of contamination.

Conclusion

The combination of the OtaCLEAN™ SMART and automated sample preparation enables fast, reproducible, consistent and sensitive analysis of ochratoxin A in coffee, but also in other matrices. High sample throughput to enable efficient and timely quality control, thus reducing production costs and analytical costs to a minimum.

The following *LCTech Products* were used:

13351	**OtaCLEAN™ SMART**
12668	**FREESTYLE SPE**
13691#AB	**ThermELUTE™**