



Fully automated mycotoxin analysis of peppers

From the raw extract to the chromatogram



EU Regulation 1370/2022 has adjusted the limits for ochratoxin A, leading to an increased demand for more sensitive analytics and rising sample numbers. This poses a challenge for sample preparation as it needs to be robust and reproducible to cope with the high sample volume and heterogeneity of sample materials.

The OtaCLEAN™ SMART columns in combination with the FREESTYLE ThermELUTE™ sample clean-up system offer efficient sample clean-up and highly sensitive analysis. The FREESTYLE ThermELUTE™, with the possibility of interfacing to any analyser, offers highly sensitive analytics not only for ochratoxin A, but also for the toxins aflatoxin B/G, aflatoxin M1 and zearalenone. Chilli and peppers are frequently found among the contaminated matrices and are particularly problematic in clean-up and chromatographic analysis due to the secondary constituents.

Product-Highlight

High matrix compatibility, high flow rates and short processing times, efficient clean up and best sensitivity make **OtaCLEAN™ SMART** and the **FREESTYLE ThermELUTE™** outstanding specialists in mycotoxin analysis.

- High loading capacity (100 ng OTA)
- Small sample volumes through thermal elution
- Best matrix compatibility - universally applicable (baby food - food - spices - animal feed)

Processing Protocol

Extract paprika, in this case a FAPAS QC material (T04389QC) was used as exemplary matrix. Add 10 g homogeneous matrix with 1 g sodium chloride, 50 mL methanol/water (80/20 (v/v)) and 25 mL n-hexane and extract for 10 minutes.

After extraction, centrifuge at 3000 x g for 5 minutes. From the n-hexane-free layer (bottom layer), remove and mix 2 mL with 12 mL of PBS buffer containing 8 % Tween 20. Place the sample in a sample vial and clean with the FREESTYLE ThermELUTE™ using the OtaCLEAN™ SMART column before automatic injection into the HPLC.



FREESTYLE ThermELUTE™

OtaCLEAN™ SMART

The entire processing procedure consists of the following steps, which are automatically mapped in FREESTYLE after appropriate parameterisation.

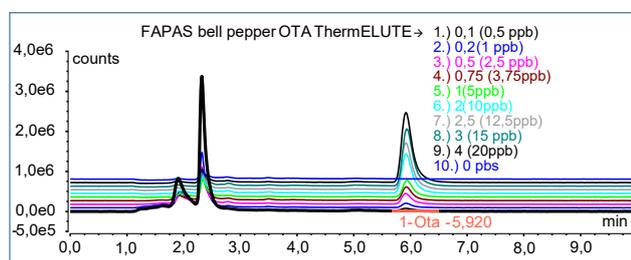
- Loading: 2.8 mL (corresponds to 0.08 g matrix equivalent) (flow rate 1.5 mL/min)
- Washing of the column: 2 mL deionised water (flow rate 1.5 mL/min)
- Elution with HPLC water by thermal elution in the FREESTYLE ThermELUTE™ 98 °C, 6 minutes.
- Automatic injection of the eluate into the HPLC.



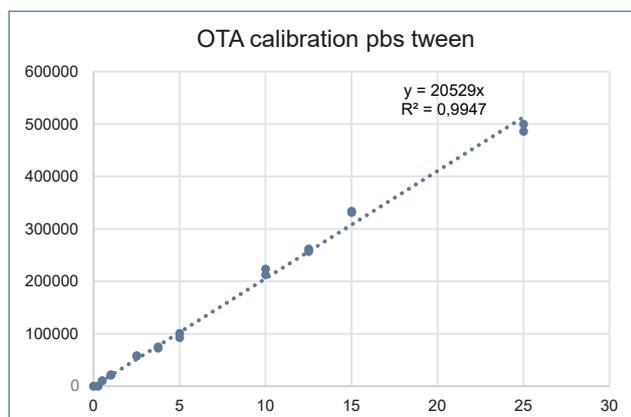
Analytic

HPLC	isocratic
Column oven	40 °C
Separation column	RP EC 125/3 nucleosil 120-3 C-18
Flow rate	0.6 mL/min
Solvent (isocratic)	HPLC-water/methanol/acetonitrile+1 % acetic acid (40/55/5 (v/v/v))
Fluorescence detection	without derivatisation
Excitation wavelength	335 nm
Emission wavelength	465 nm

Chromatograms



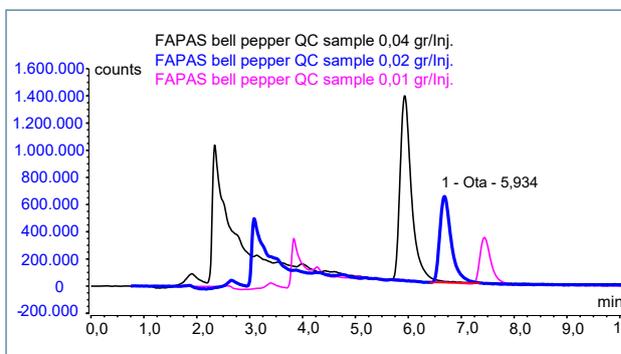
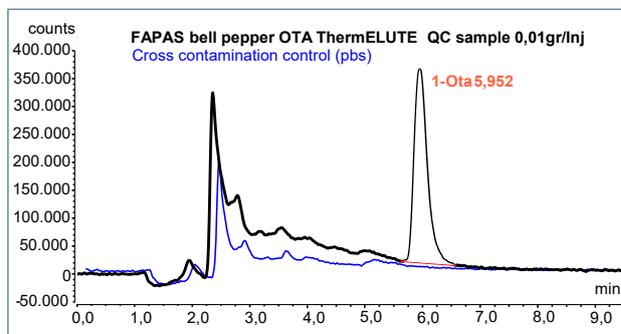
Chromatograms of the calibration concentrations (0-20 ppb), an extension of the calibration to lower and higher toxin concentrations is possible.



The calibration line covers the range 0-25 ppb linearly (correlation coefficient 0.9947).

The sample materials were analysed chromatographically and the OTA content determined. According to the certificate, the material should contain a content of 7.4-18.9 ppb. A concentration of 16.2 ppb was determined with an error of 1.4 %. With repeated processing, the content could always be confirmed. Both the tests regarding matrix tolerance showed the robust, precise and reproducible processing of the reference material sample in the FREE-STYLE ThermELUTE™ (see chromatogram).

The system has prevented cross-contamination (see chromatogram). The consistent cleaning of the system by the software-controlled processes showed no carry-over of matrix interferences or sample residues. Minimum workload for maximised sample throughput.



Conclusion

The OtaCLEAN™ SMART immunoaffinity column is suitable for the analysis of ochratoxin in various matrices, including spices such as peppers and chilli. Reproducible good results have been obtained in accordance with certified reference materials. The tolerance to these matrices also allows analysis in the baby food range. Especially due to the technology of thermal elution, the large volume injection for the mycotoxin range is a gain in measurement sensitivity and, in combination with the fully automated processing, an asset for any analytical laboratory. Compatibility with any HPLC-FLD or LC system allows universal use in food and feed testing laboratories.

The following LCTech products have been used::

- 13346 / 13351 OtaCLEAN™ SMART
- 12663 / 12668 / 13691 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

