SOLUTIONS BY LCTech

Fully Automated Mycotoxin Analysis From Extract to Chromatogram

at a Sensitivity in the ppt or ppq Level Using not only LC-MS/MS but HPLC-FLD

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Mycotoxin analysis needs time-intensive clean-up or high-end analytical devices, especially, if a high sensitivity i.e. for baby food is required. A complete automation using thermal denaturation technology and SMART columns reduces not only method working steps but also time and costs per sample. At the same time a sensitivity in the ppq level is gained in combination with HPLC-FLD.

Fig. 1: FREESTYLE ThermELUTE™

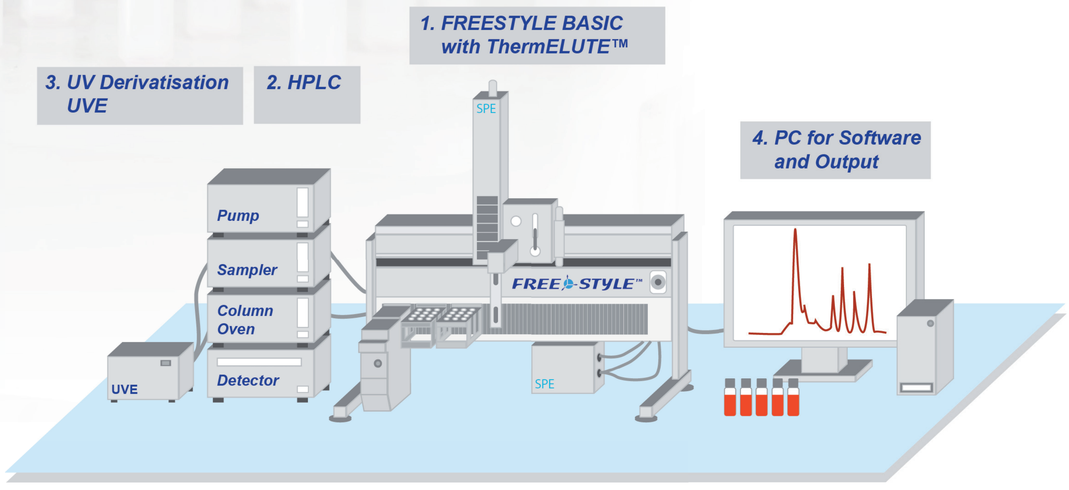


Fig. 2: Configuration with FREESTYLE ThermELUTE™

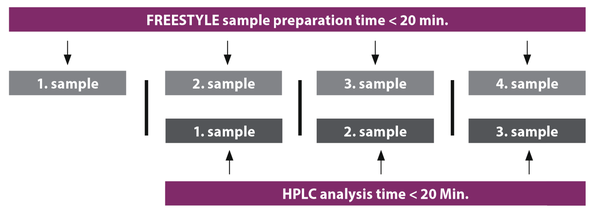


Fig. 3: Processing Time FREESTYLE ThermELUTE™

Method Description for Automated Processing via ThermELUTE™

Samples were extracted with methanol/water (80/20 v/v)) and filtered. Fatty or oil containing matrices (e.g. spices) were treated with n-hexane for defatting. The dilution of the sample was done acc. to the extraction and clean-up protocols of the SMART columns (fig. 9). 2.8 – 20 mL of the sample, representing 0.08 – 10 g matrix equivalents, were loaded fully automated via the robotic system FREESTYLE ThermELUTE™ (fig. 1) onto the SMART column (fig. 9) with a max. flow rate of 3 mL/min. After a washing step, in order to remove matrix interferrences, the column was eluted via ThermELUTE™ technology for denaturation of the antibodies. The eluate was quantitatively and directly injected into the HPLC-FLD (fig. 2). During the HPLC analysis of the previously prepared sample, FREESTYLE ThermELUTE™ processes the next sample in parallel (fig. 3).

Reproducible Results with FREESTYLE ThermELUTE™

LOD / LOQ

Analyte	LOD (ppt [ng/kg])	LOQ (ppt [ng/kg])
Aflatoxin B1	7	21
Aflatoxin B2	2	6
Aflatoxin G1	8	23
Aflatoxin G2	2	7
Aflatoxin M1	0.7	2.2
Ochratoxin A	10	31
Zearalenone	1	2.6

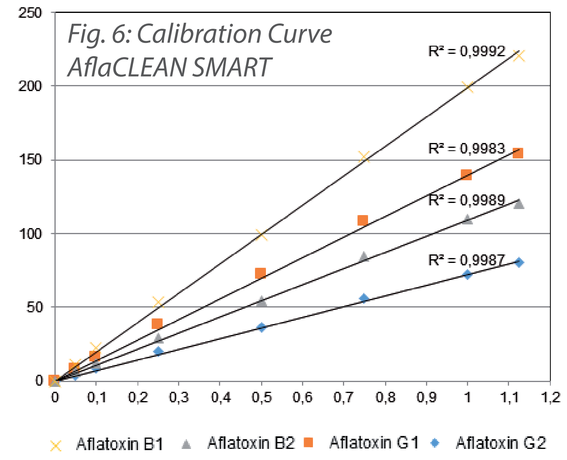
Fig. 4: LOD/LOQ Results for Different Analytes

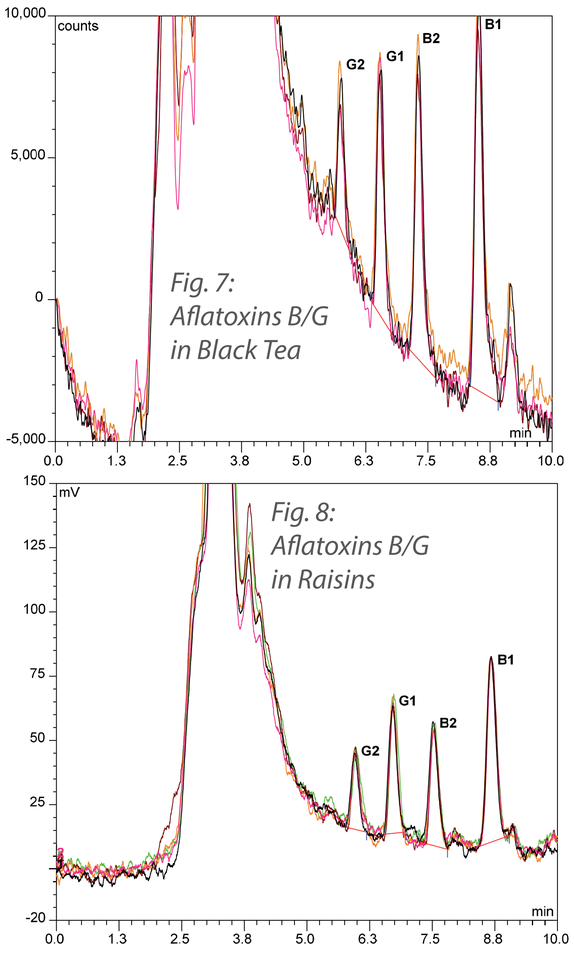
Recovery Rates SMART-Columns

Matrix	Recovery Rates for Aflatoxins B/G, M1, and Ochratoxin A in %			
Aflatoxin B/G (AflaCLEAN SMART)				
	B1	B2	G1	G2
Pistachio	99	94	95	81
Curcuma	96	107	102	104
Corn	101	95	100	85
Black Tea*	94	91	97	83
Raisins*	96	94	97	91
Aflatoxin M1 (AflaCLEAN M1 SMART)				
Milk	96			
Raw Milk	90			
Milk Powder	98			
Ochratoxin A (OtaCLEAN SMART)				
Coffee	98			
Chili	98			
Corn	101			

* Results in the Measuring Range of Baby Food

Fig. 5: Recovery Rates for Difficult Matrices





AflaCLEAN SMART Calibration Curve

Fully Automated via FREESTYLE ThermELUTE™

The calibration curve shows a very high linearity and good correlation over a wide measuring range using the immunoaffinity columns AflaCLEAN SMART (fig. 9) processed via FREESTYLE ThermELUTE™.

By variing the matrix load, the calibration curve can be used for individual matrices from baby food up to animal feed or human food.

Fig. 9: SMART-Columns with Tip

Black Tea

Fully Automated via FREESTYLE ThermELUTE™

Chromatograms representing black tea (0.08 g) spiked with 0,25 ppb (µg/kg) total aflatoxin ≙ 0.1 ppb B1.

The overlay of the five chromatograms clearly shows the reproducibility of the results for black tea.

Raisins

Fully Automated via FREESTYLE ThermELUTE™

Chromatograms representing raisins (0.28 g) spiked with 0.25 ppb (µg/kg) total aflatoxin ≙ 0.1 ppb B1.

The overlay of the chromatograms clearly demonstrates the reproducibility of the results for raisins.

Conclusion

FREESTYLE ThermELUTE™ enables a complete automation from raw extract to chromatogram. No other system shows a capable reduction of workload, applicative flexibility, and sensitivity at trace levels, respectively (fig. 4). Without any time consuming manual sample preparation steps e.g. evaporation of the samples, the required maximum levels for baby food are measured and monitored with best recoveries and excellent performance specifications (fig. 4-8).

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