

SOLUTIONS BY



## **FREESTYLE ThermELUTE™**

- Excellent Results
- Best Reproducibility
- No Cross-contamination

## FREESTYLE ThermELUTE™

### General

The robotic system FREESTYLE ThermELUTE™ in combination with any HPLC with FLD allows the fully automated mycotoxin analysis for the aflatoxins B1, B2, G1, G2 and M1 as well as ochratoxin A.

The system needs significantly less sample and solvents due to the miniaturization of the sample preparation process. In addition the process time is drastically reduced. Cross-contamination is reliably excluded as for each sample an individual immunoaffinity column is used.



*SMART immunoaffinity columns*

Within the ThermELUTE™ module the toxin antibody bond is broken by thermal denaturation. The toxins in form of a large-volume, aqueous eluate are completely eluted into the HPLC sample loop via partial filling.

This unique technology achieves extremely low detection limits in the lower ppt-range without additional efforts, such as evaporation.

## **FREESTYLE ThermELUTE™** **Advantages at a Glance**

- Fully automated mycotoxin analysis
- Suitable for almost every matrices
- Very high sensitivity in the lower ppt-range
- High sample throughput (> 70 samples / day; 120 samples / weekend)
- Unbeatable fast processing
- Reproducible results
- Excellent chromatography
- Only very low sample volumes needed

The following pages will show you the results of matrices with a very difficult sample preparation. We highlight the aspect of the reproducibility of these results processed via FREESTYLE ThermELUTE™ and show that no cross-contamination from one sample to the subsequent occurs.

## **FREESTYLE ThermELUTE™** **Reproducibility of the Results**

### Procedure

Five samples of the same matrix are extracted and filtrated corresponding to the official requirements. An aliquot is processed by the robotic system FREESTLYE ThermELUTE™. For each sample an individual immunoaffinity column, AflaCLEAN SMART, OtaCLEAN SMART or AflaCLEAN M1 SMART, is used (depending on the analyzed toxin and the matrix).



FREESTYLE ThermELUTE™ module

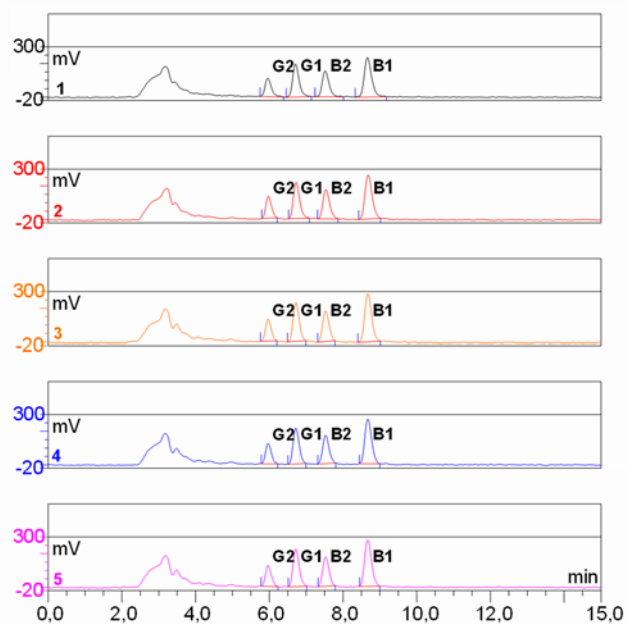
## Reproducibility of the Results

### Matrix: Figs

20 g figs are extracted with 80/20 +1 g sodium chloride and 50 mL n-hexane, filtrated and diluted with PBS.

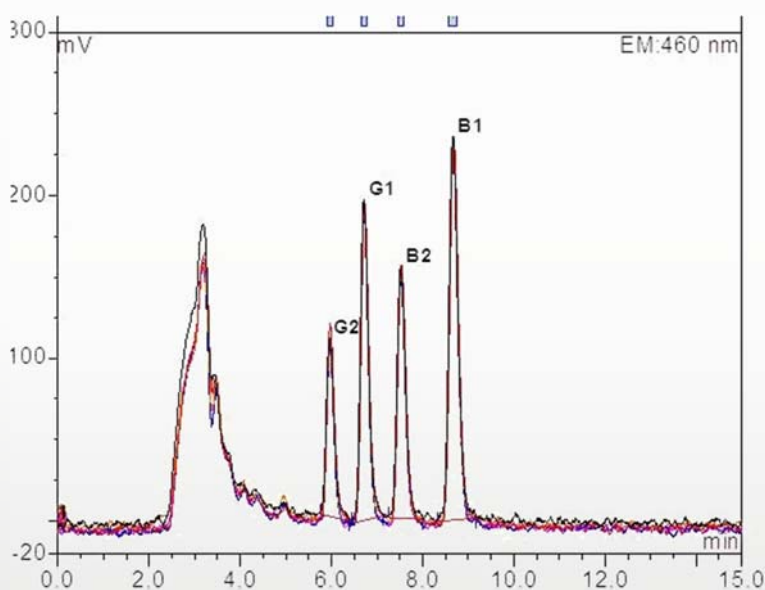
After renewed filtration 1.5 mL with 13.5 mL 11.2 % methanol are diluted in PBS and 10 mL (represent 0.028 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 10 ppb total aflatoxin (B1/G1 each 4 ng/g; B2/G2 each 1 ng/g). Five consecutive chromatograms have been tested for the chromatographical comparability.



Figs (0.028 g matrix), spiked with 10 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the five chromatograms** shows clearly the reproducibility of the results.



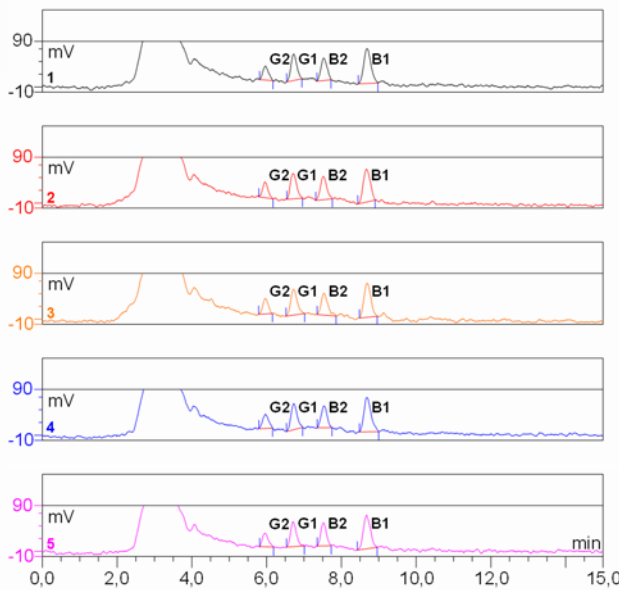
## Results for strong regulated measurement ranges, such as i.e. baby food

### Matrix: Figs



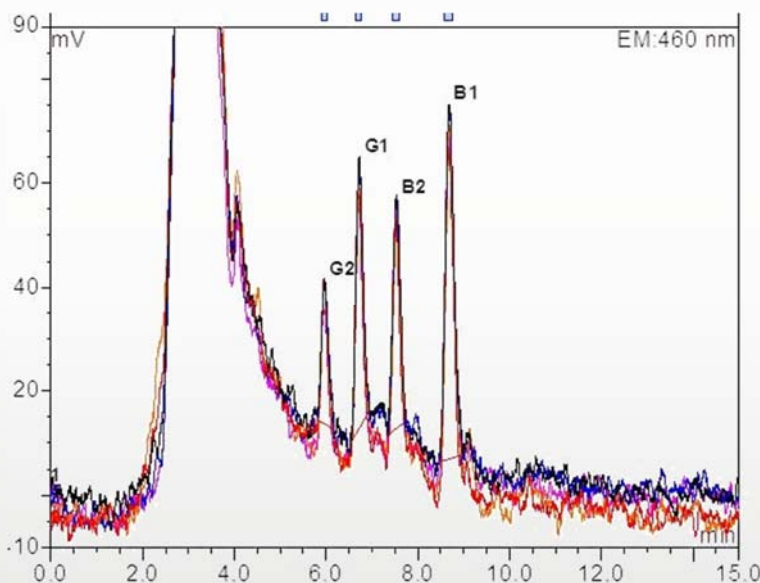
The matrix was spiked with 0.25 ppb total aflatoxin (B1/G1 each 0.1 ng/g; B2/G2 each 0.025 ng/g). 10 mL (represent 0.28 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 0.25 ppb total aflatoxin (B1 0.1 ppb). Five consecutive chromatograms have been tested for the chromatographical comparability.



Figs (0.28 g), spiked with 0.25 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the chromatograms** demonstrates clearly the reproducibility of the results for the strong regulated measurement range too.



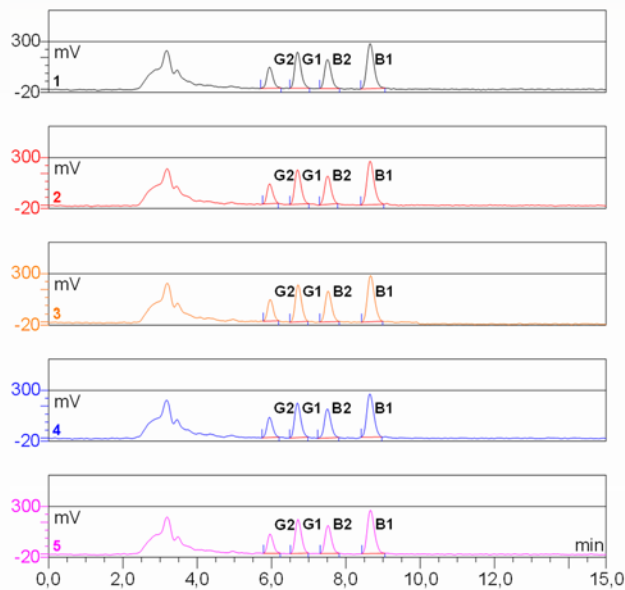
## Reproducibility of the Results

### Matrix: Raisins

20 g raisins are extracted with 80/20 +1 g sodium chloride und 50 mL n-hexane, filtrated and diluted with PBS.

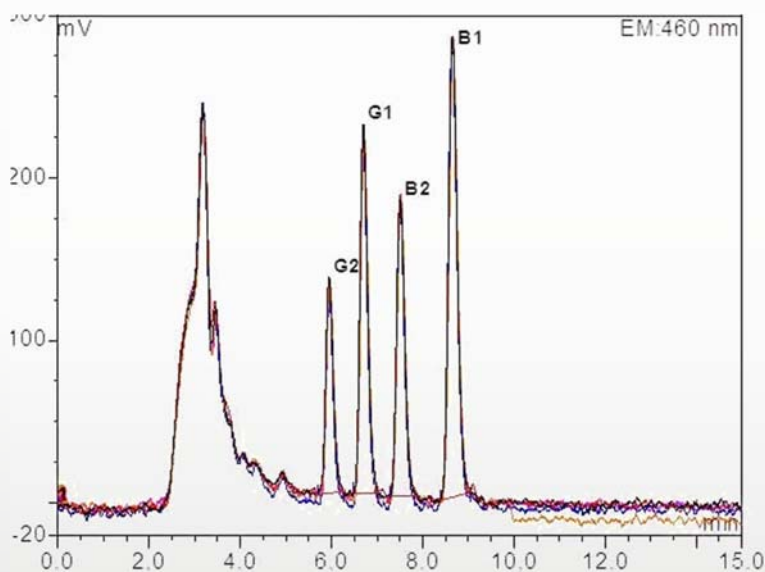
After renewed filtration, 1.5 mL are diluted with 13.5 mL 11.2 % Methanol in PBS and 10 mL (represent 0.028 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 10 ppb total aflatoxin (B1/G1 each 4 ng/g; B2/G2 each 1 ng/g). Five consecutive chromatograms have been tested for the chromatographical comparability.



Raisins (0.028 g), spiked with 10 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the five chromatograms** shows clearly the reproducibility of the results, also for the samples of raisins.



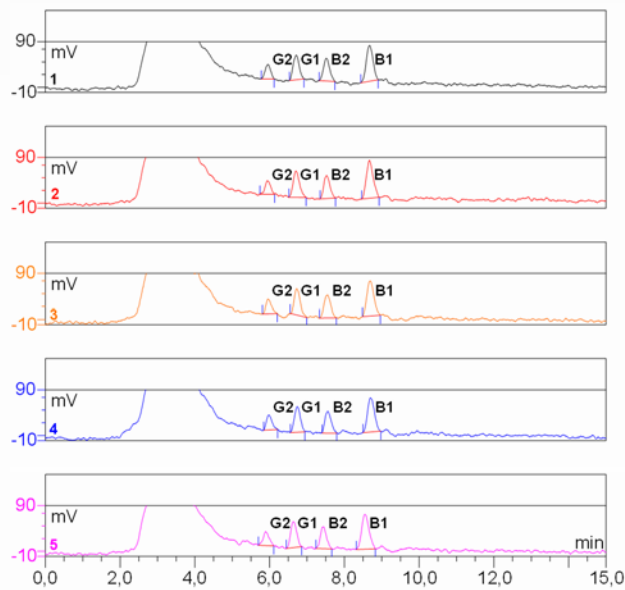
## Results for strong regulated measurement ranges, such as i.e. baby food

### Matrix: Raisins



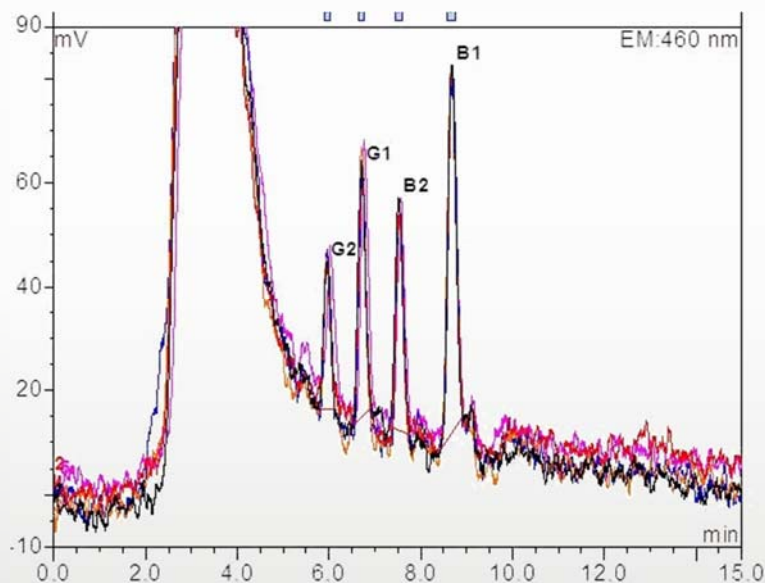
The matrix was spiked with 0.25 ppb total aflatoxin (B1/G1 each 0.1 ng/g; B2/G2 each 0.025 ng/g). 10 mL (represents 0.28 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 0.25 ppb total aflatoxin (B1 0.1 ppb). Five consecutive chromatograms have been tested for the chromatographical comparability.



Raisins (0.28 g), spiked with 0.25 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the chromatograms** demonstrates clearly the reproducibility of the results for the strong regulated measurement range too.



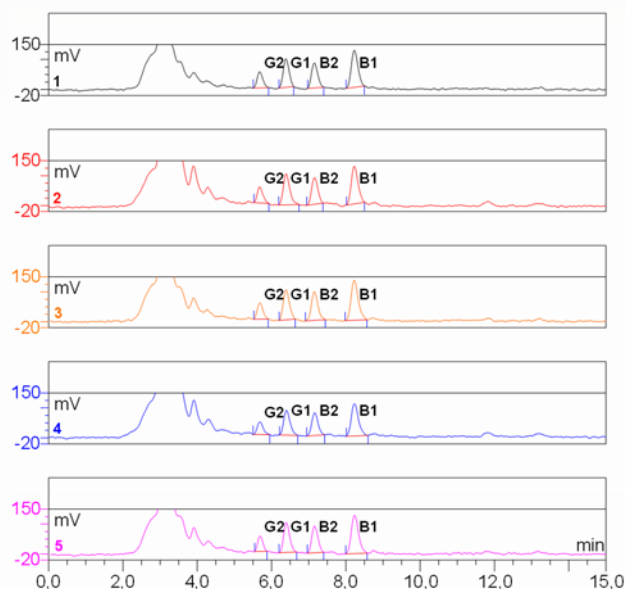
## Reproducibility of the Results

### Matrix: Parsley

5 g parsley are extracted with 80/20 +1 g sodium chloride and 50 mL n-hexane, filtrated and diluted with PBS.

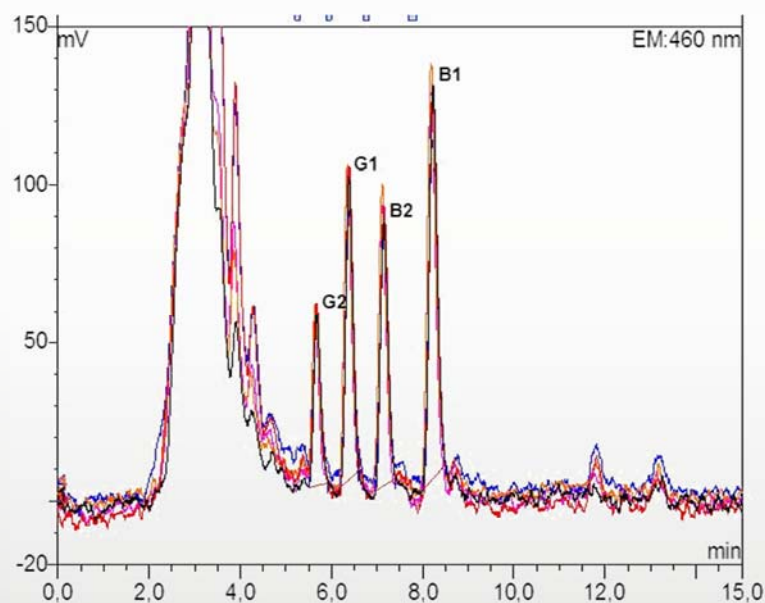
After renewed filtration 2 mL are diluted with 12 mL PBS (8 % Tween20) and 2.8 mL (represent 0.02 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 10 ppb total aflatoxin (B1/G1 each 4 ng/g; B2/G2 each 1 ng/g). Five consecutive chromatograms have been tested for the chromatographical comparability.



Parsley (0.02 g), spiked with 10 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the five chromatograms** demonstrates clearly the reproducibility of the results, also for parsley samples.





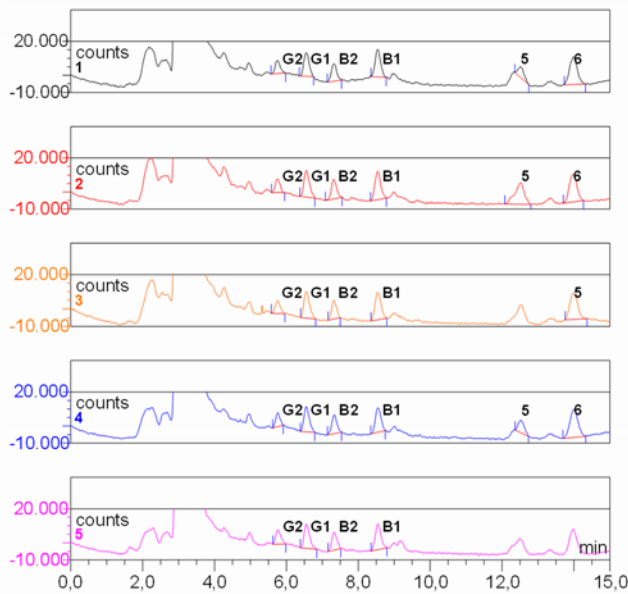
## Results for strong regulated measurement ranges, such as i.e. baby food

### Matrix: Parsley



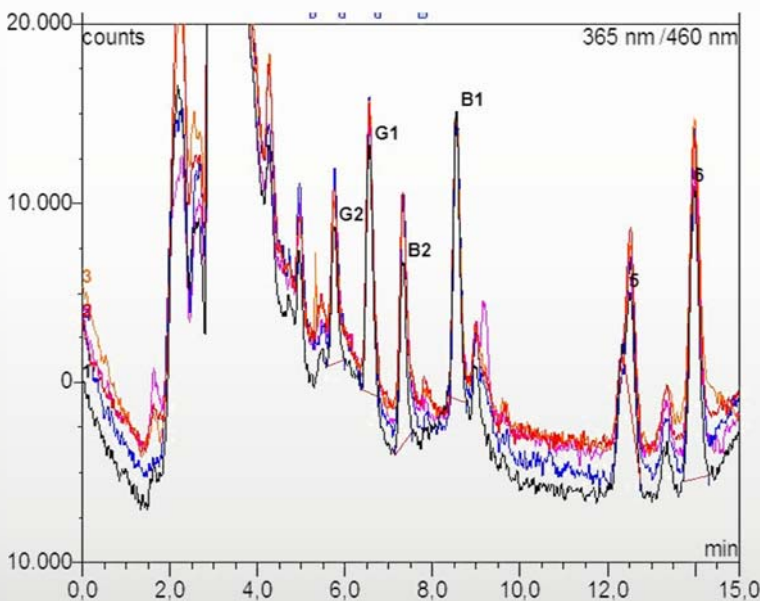
10 g parsley were extracted with 80/20 (100 mL) and 50 mL n-hexane and 1g sodium chloride. The raw extract was filtrated and 2 mL were diluted with 12 mL PBS, containing 8 % Tween20. 2.8 mL (represent 0.04 g matrix equivalent) was processed.

Prior to this the matrix was spiked with 0.25 ppb total aflatoxin (B1 0.1 ppb). Five consecutive chromatograms have been tested for the chromatographical comparability.



Parsley (0.04 g), spiked with 0.25 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the chromatograms** shows clearly the reproducibility of the results for the strong regulated measurement range.



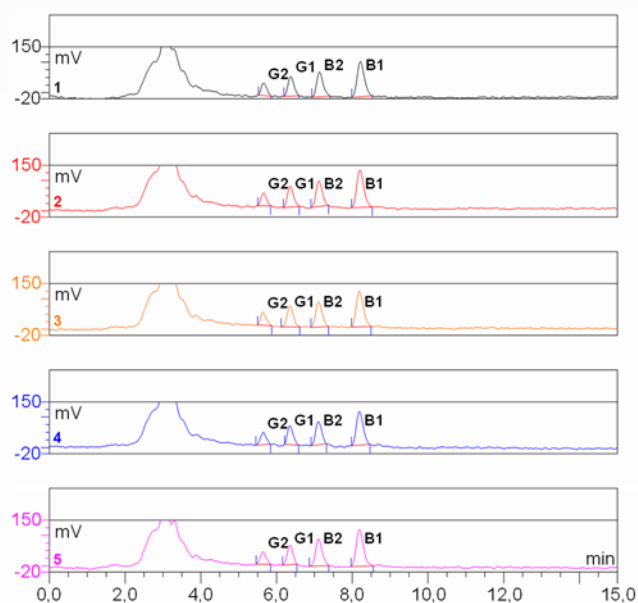
## Reproducibility of the Results

### Matrix: Black Tea

5 g black tea (Earl Grey) were extracted with 80/20 +1 g sodium chloride and 50 mL n-hexane, filtrated and diluted with PBS.

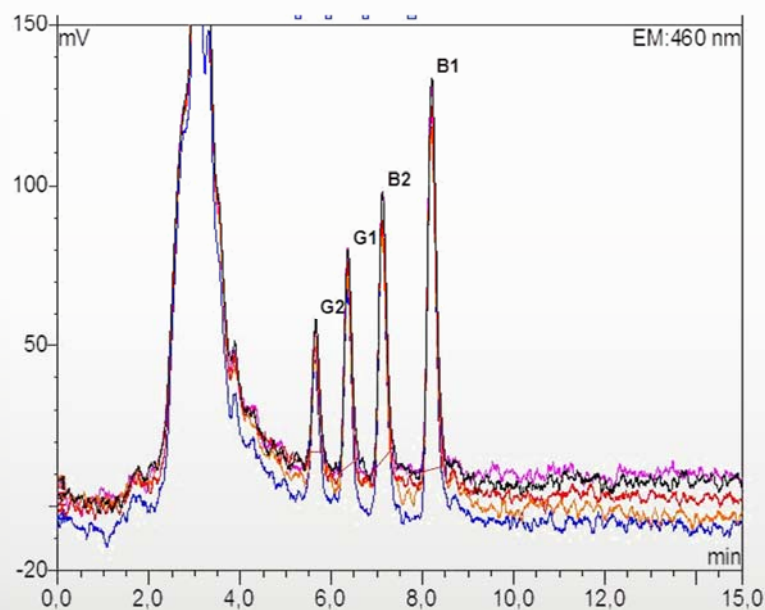
After renewed filtration 2 mL are diluted with 12 mL PBS (8 % Tween20) and 2.8 mL (represent 0.02 g matrix equivalent) are processed via FREESTYLE ThermELUTE™.

Prior to this the matrix was spiked with 10 ppb total aflatoxin (B1/G1 each 4 ng/g; B2/G2 each 1 ng/g). Five consecutive chromatograms have been tested for the chromatographical comparability.



Black tea (0.02 g), spiked with 10 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

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



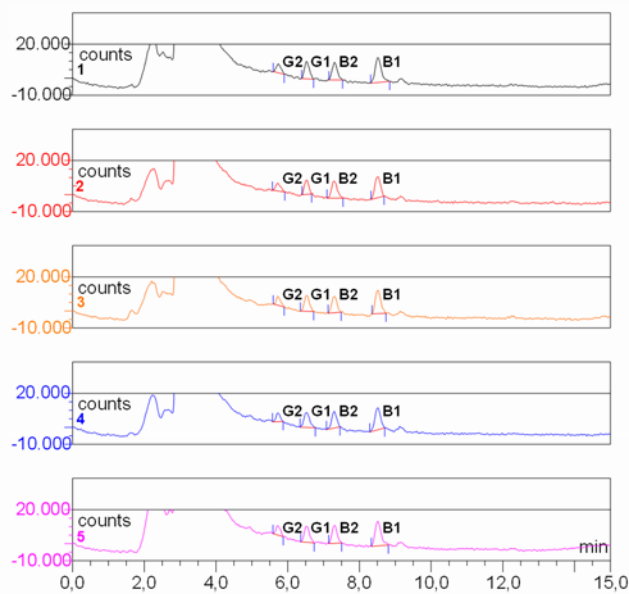
## Results for strong regulated measurement ranges, such as i.e. baby food

### Matrix: Black Tea

10 g black tea (Earl Grey) were extracted with 80/20 (100 mL) and 50 mL n-hexane and 1g sodium chloride. The raw extract was filtrated and 2 mL were diluted with 12 mL PBS, containing 8 % Tween20. 2.8 mL (represent 0.04 g matrix equivalent) was processed.

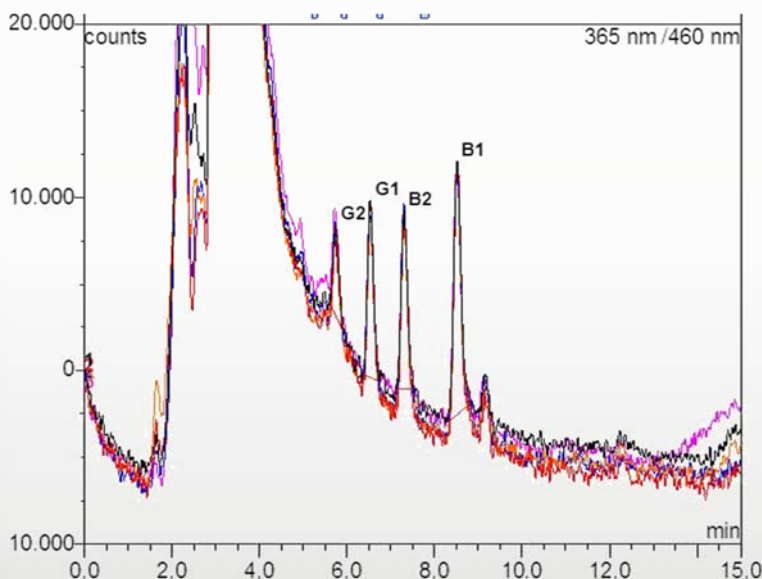


Prior to this the matrix was spiked with 0.25 ppb total aflatoxin (B1 0.1 ppb). Five consecutive chromatograms have been tested for the chromatographical comparability.



Black tea (0.04 g), spiked with 0.25 ppb ( $\mu\text{g}/\text{Kg}$ ) total aflatoxin

The **overlay of the chromatograms** shows clearly the reproducibility of the results for the strong regulated measurement range too.



## **FREESTYLE ThermELUTE™** **Amounts of Matrix for the Injection**

Only very low amounts of matrix are loaded onto the immunoaffinity column due to the miniaturization of the complete sample preparation process and the direct injection from the ThermELUTE™ module into the sample loop of the HPLC system.

According to 2.8.18 determination of aflatoxin B1 in herbal drugs (European Pharmacopoeia 2.8.18, Core Manual 2014) 0.25 g are loaded onto the immunoaffinity column and 0.025 g of the matrix equivalent are finally injected according to the requirement. The limits are at 4 ppb (addition of G2/G1/B2/B1). However 40 mL of the sample are loaded onto the immunoaffinity column and 0.5 mL of the diluted eluate (5 mL), which means 10 %, are loaded.

Parsley: 0.02 g are loaded onto the SMART immunoaffinity columns via FREESTYLE and are with quantitative transfer directly injected into the HPLC system after the denaturation.

The data shown on the previous pages prove that the measurement of 0.1 ppb aflatoxin B1 is possible (shown is 0.25 ppb total aflatoxin).

This clearly demonstrates that by using the FREESTYLE ThermELUTE™ measurements and quantifications are happening at least **16 times below** the regulated limits.



Gripper takes adapter.



Adapter takes column.

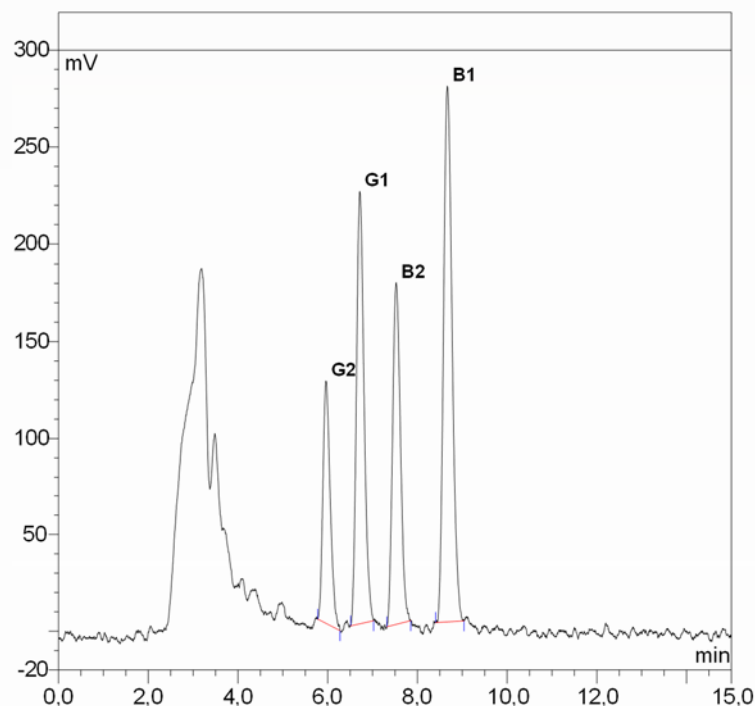


Column is injected into the ThermELUTE™ module.

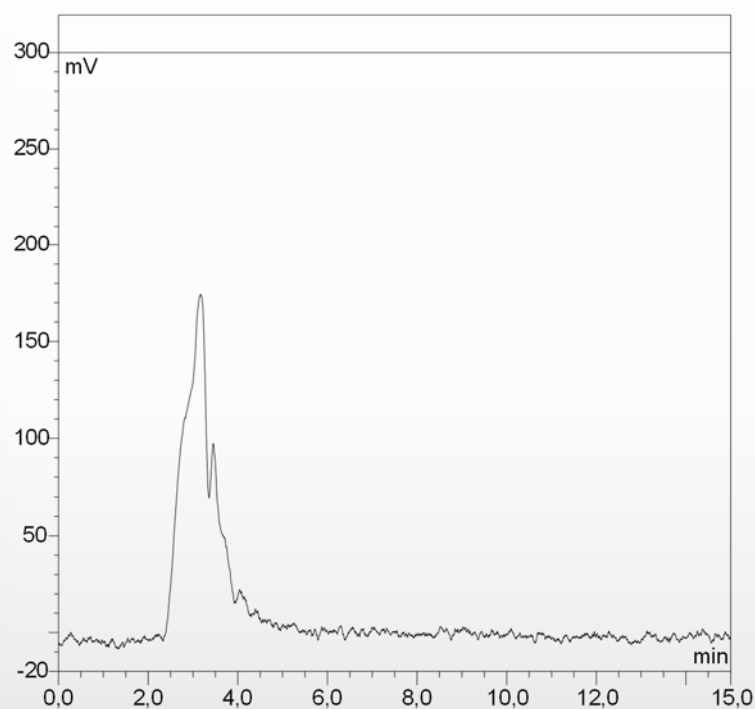
## FREESTYLE ThermELUTE™ No Cross-contamination

The following chromatograms show that cross-contamination is reliably avoided processing the mycotoxin samples via the FREESTYLE ThermELUTE™.

A sample spiked with 10 ppb total aflatoxin was processed by the robotic system. After this a blind sample was processed.



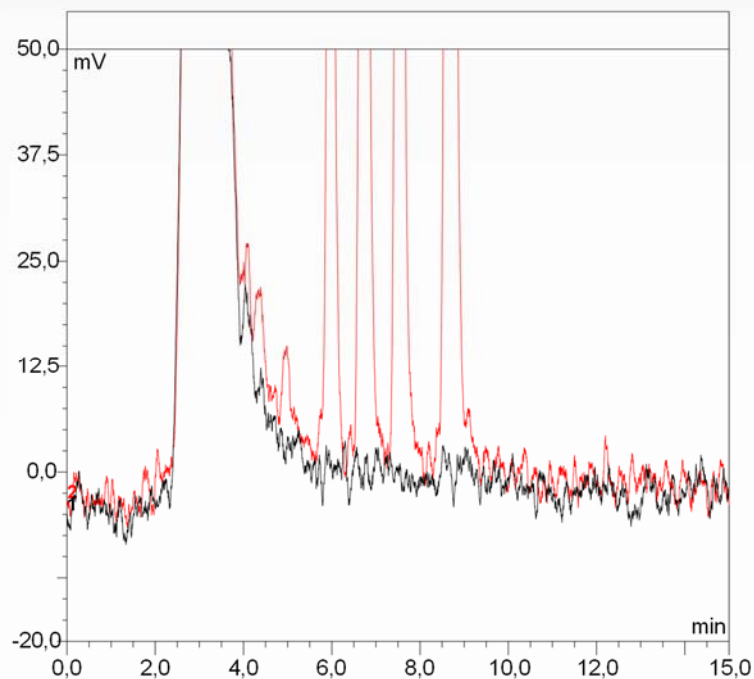
Figs, spiked with 10 ppb total aflatoxin



Blind sample

# FREESTYLE ThermELUTE™ | AS002

The following overlay of the chromatograms demonstrates again that there is **no cross-contamination** when processing the samples via FREESTYLE ThermELUTE.



Figs, spiked with 10 ppb total  
aflatoxin (red)  
Blind sample (black)



FREESTYLE ThermELUTE™

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FREESTYLE ThermELUTE™ Videos**

Don't miss our new video about the robotic system FREESTYLE ThermELUTE™. It shows you the fully automated processing of mycotoxin samples: fast, reliable and reproducible! You find it on our website [www.LCTech-online.com](http://www.LCTech-online.com).