

SOLUTIONS BY



# Determination of Aflatoxin M1 via FREESTYLE ThermELUTE™ with Online HPLC-Measurement

## Determination of Aflatoxin M1 via FREESTYLE ThermELUTE™ with Online HPLC-Measurement

### Principle of the Method

Aflatoxin M1 is a highly toxic mycotoxin which gets formed from aflatoxin B1 within animals consuming it in their feed. The toxin (fig. 1) can be found in milk, dairy products, meat and eggs. 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) 165/2010.

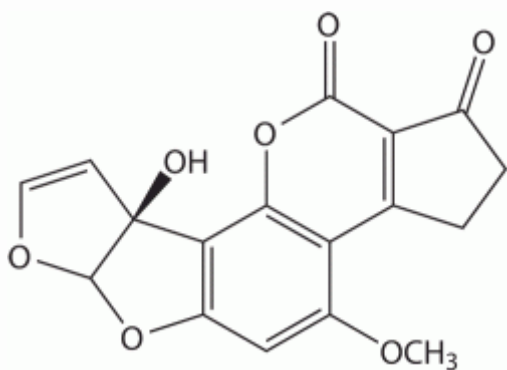


Fig. 1: Structure of Aflatoxin M1

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 AflaCLEAN M1 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 AflaCLEAN M1 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. AflaCLEAN M1 SMART  | P/N 14246 (100 pcs/pck) or<br>P/N 14248 (1000 pcs/pck) |
| 11. Injection needle  | P/N 13544 (1000 pcs/pck)                               |
| 12. Methanol p.a.   |  |
| 13. Water p.a.  |  |
| 14. Aflatoxin M1-Eluator  |  |
| 15. Standard laboratory equipment including HPLC with fluorescence detector |  |



Gripper takes adapter.



Adapter takes columns.



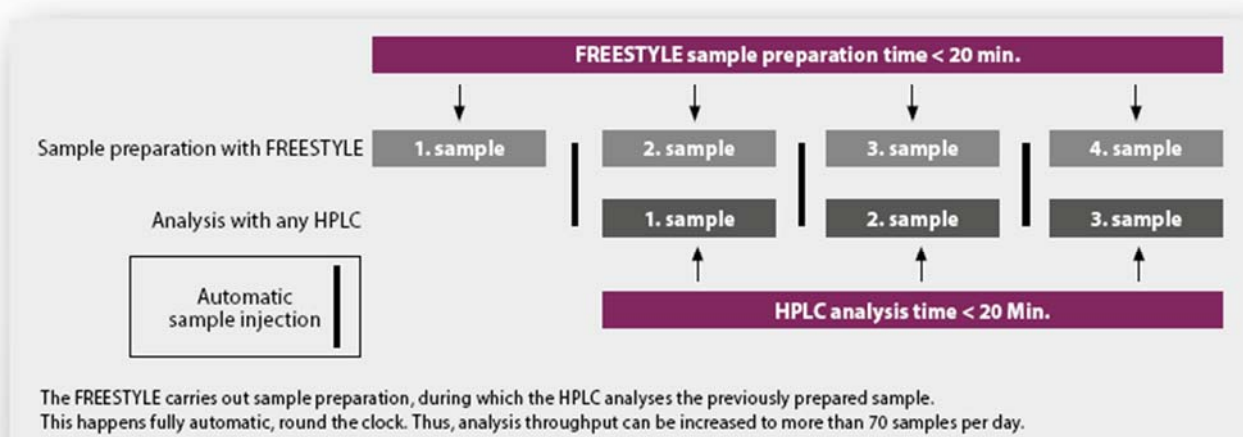
Columns is transferred into the ThermELUTE™ module.

## Parameterization of the FREESTYLE ThermELUTE™ Method



LC Tech FreeStyle - Report on Methods: ThermElute      Date: 02.12.2014    Time: 12:13:10

<b>Name:</b> Aflatoxin_M1_Milch.tel		
Column:	AflaCLEAN_SMART.col	
Initial System Cleaning Port:	9	40/60 MeOH/Water
Sample Volume:	20 ml	Bottle type: Type1@60 ml
Flow rate sample:	1 ml / min.	
Port for 1. cleaning step after loading	9	40/60 MeOH/Water
Port for 2. cleaning step after loading	1	HPLC Water
Volume washing solution:	4 ml	
Flow rate washing solution:	1 ml / min.	
Port for 2. Washing solution:	1	HPLC Water
Elution positioning volume:	810 µl	
	Standard = 600 µl	
Flow rate eluting solution:	1.5 ml / min.	
Eluting solution:	11	Elutionsolvent
Temperature heating:	98 °C	
Heating time:	360 sec.	
Cooling time:	0 sec.	
Cleaning Step - Solvent from Port:	9	40/60 MeOH/Water
Wait minimum process time for a longer HPLC measurement		20 min.
Use pressure limitation function during loading and washing		
Pressure limit for syringe pump		80 digits
If pressure limit is triggered:		
Time from start of sample to injection for HPLC		20 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 Aflatoxin M1 for various matrices:

Matrix	Matrix Load [g]	Recovery [%]
Milk	10	96
Raw milk	10	90
Milk powder	1,0	98

The samples were spiked with 80 ppt (80 ng/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: 1.2mL/min, acetonitrile / water / methanol (15/60/30 (v/v/v))

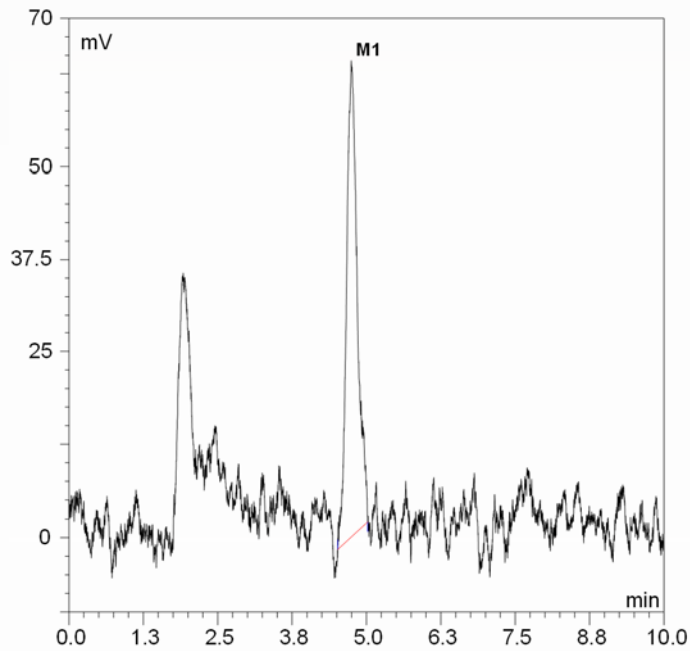
Separation column: RP C18 (LCTech P/N 10522)

Fluorescence detection

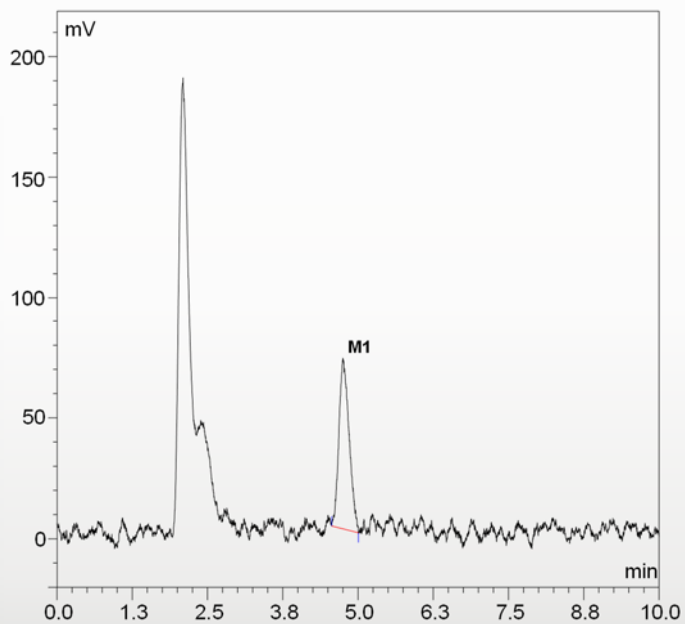
Excitation wavelength: 365nm

Emission wavelength: 435 nm

Chromatography of 0.8 ng/400  $\mu$ L aflatoxin M1 standard

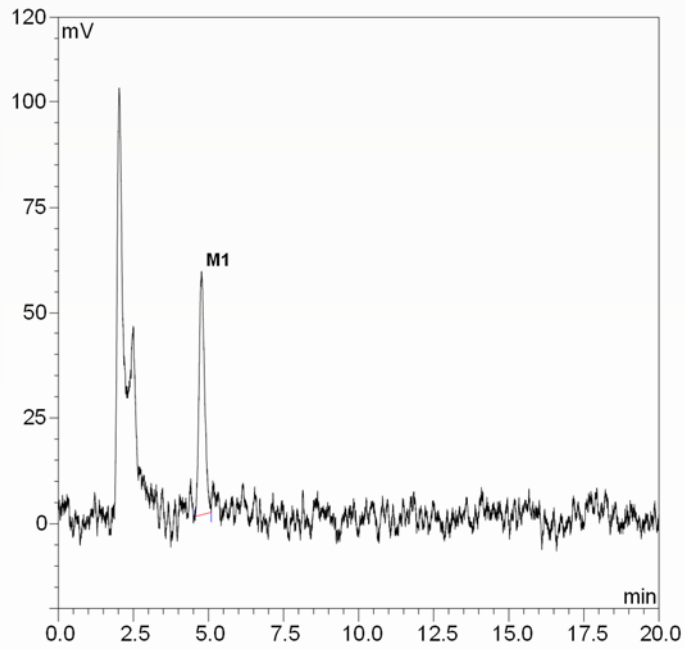


Chromatography of aflatoxin M1 0.8 ng in 10 mL milk UHT (80 ppt) extracted, according to the protocol for UHT and raw milk.

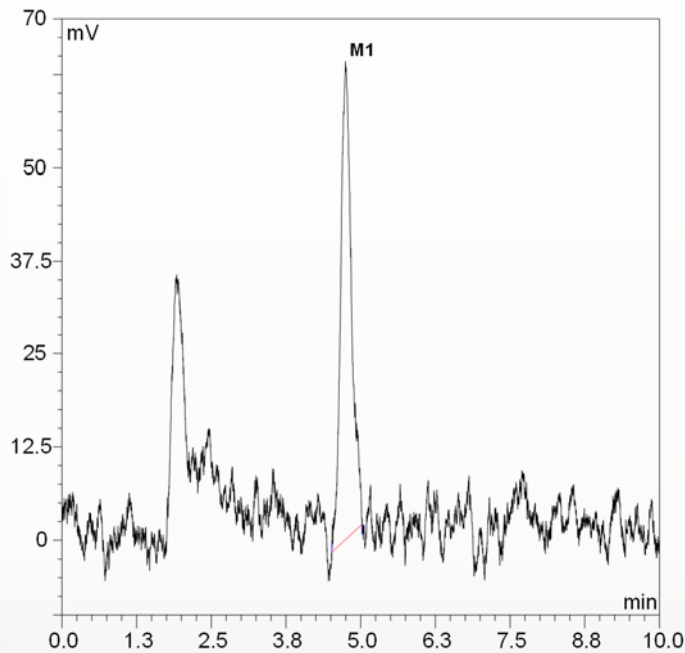


# APPLICATION NOTE | AN0006

Chromatography of aflatoxin M1, extracted from raw milk according to the official AOAC 2000.08 method, spiked with 80 ppt aflatoxin M1.



Chromatography of Aflatoxin M1, extracted from milk powder according to the described protocol and spiked with 80 ppt aflatoxin M1.





## Literature and Regulations

- AOAC Official Method 2000.08  
Aflatoxin M1 in Liquid Milk.  
Immunoaffinity Column by Liquid Chromatography.  
First Action 2000
- DIN EN ISO 14501:2008-01  
European norm for extraction and analysis of aflatoxin M1 in milk and milk powder
- EC 165/2010  
COMMISSION REGULATION (EU) No 165/2010  
of 26 February 2010  
amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants  
in foodstuffs as regards aflatoxins
- H. Marina Martins, RPCV (2007) 102 (563-564) 321-325  
Aflatoxin M1 determination in cheese by immunoaffinity column clean-up coupled to  
high-performance liquid chromatography



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