



Aflatoxin M1 in Milk

Cleaned-up with *AflaCLEAN M1 SMART*



Transmission of aflatoxin from feed into milk

Due to the contamination of animal feed by aflatoxins, especially aflatoxin B1, there are repeated transmissions of the derivative aflatoxin M1 in milk and milk products, such as cheese, yoghurt and ice cream. Due to the toxicity, correspondingly strict regulations have been set for the maximum tolerated levels of aflatoxin M1 (EC1881/2006).

The determination limits to be complied with can only be analytically ensured by large matrix quantities, but there are possibilities to achieve the measurement sensitivity through consistent downscaling of all processes, without loss of performance and with less time saving.

Clean-up of Aflatoxin M1 in milk as food

Small + Fast + Economical = SMART

LC Tech has developed the immunoaffinity column AflaCLEAN M1 SMART for sample preparation within routine analysis by means of HPLC with fluorescence detection or LC-MS. It is specially designed for the clean-up of aflatoxin M1. The sample can be analysed for aflatoxin M1 faster than „milking cows“!

Advantages at a glance

- Excellent recoveries - even with difficult matrices: Aflatoxin M1 >80 %
- Savings of 80 % solvent through miniaturisation
- Shelf life: 9 months if stored at 4 to 8 °C
- Only 3 cm big
- Suitable for automated processing

Processing Protocol

The milk is warmed to room temperature, then centrifuged at 3500 xg for 10 minutes to separate the cream. After centrifugation, the cream is carefully but completely skimmed from the milk. The milk is filtered through a pleated filter to remove solids and aggregates.

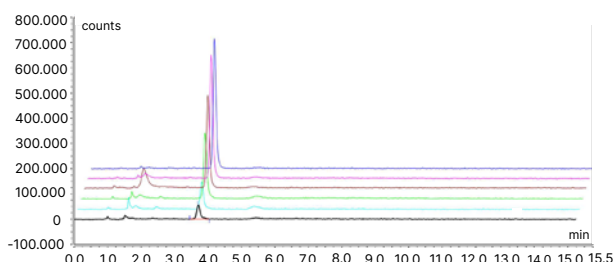
10 mL of the filtered milk is mixed with 10 mL of PBS buffer. The sample (representing 10 mL of milk) is fully loaded onto the AflaCLEAN M1 SMART immunoaffinity column (flow rate 1.5 mL min). The loaded column is

rinsed twice with 2 mL deionised water, to completely remove the milk proteins. The column is dried by a stream of air and then eluted with 400 µL methanol.

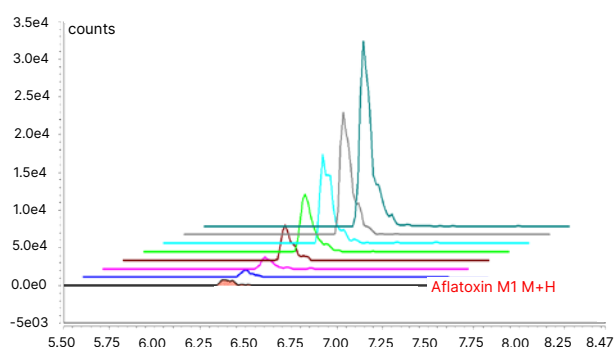
It should be noted that the methanol is allowed to act in the column bed for at least 5 minutes to ensure complete elution. The samples are measured analytically as small volume injection or adjusted to LC solvent concentration as large volume injection. Analysis by HPLC-FLD or LC-MS/MS is possible (see chromatograms).



Chromatograms



Comparison of some milk analysis samples with different aflatoxin contents (HPLC-FLD)



Comparison of some milk analysis samples with different aflatoxin contents (LC-MS/MS)

Conclusion

The analysis of milk samples for aflatoxin M1 can be guaranteed by using the AflaCLEAN M1 SMART and allows a reliable determination of the aflatoxin M1 content over a wide concentration range. AflaCLEAN M1 SMART offers an alternative to large-volume sample clean-ups through consistent downscaling.

Thus, milk samples from below the baby food limit, from aflatoxin-free to contaminations around 1.5 ppb (30 x exceeding the legal limit), can be determined safely, precisely and reproducibly in a fraction of the time (faster than „milking cows“). The clean-up procedure is compatible with your measurement analytics (HPLC-FLD or LC-MS/MS).

These LCTech products were used:

14246	AflaCLEAN M1 SMART (100 pcs/box)
10522	HPLC Column for Mycotoxins

Conditions	
HPLC	isocratic
Column Oven	36 °C
Separation Column	RP C-18 150mm ID 4,6mm P/N 10522
Flow Rate, Eluent	1.2 mL/min (60/30/15 (v/v/v)) (HPLC-water/methanol/acetonitrile)
Fluorescence Detecion	No post-column derivatisation necessary
Excitation Wavelength	365 nm
Emission Wavelength	435 nm
LC-MS/MS	gradient
Column Oven	38 °C
Separation Column	Accucore Biphenyl
Flow Rate, Eluent	0.4 mL/min (Eluent A: 98 % HPLC-water, 2 % methanol, 1 % acetic acid 96 %, 5 mm ammoniumacetate; Eluent B: 2 % HPLC-water, 98 % methanol, 1 % acetic acid 96 %, 5 mm ammoniumacetate)
MS Parameters	H-ESI 3500V; Sheath gas 40 arb.; Aux Gas 10 arb.; Sweep Gas 0 arb.; Ion transfer tube temperature 325 °C; Vaporizer temperature 350 °C, Collision Gas 1,5 arb.
Aflatoxin M1 (M+H) m/z	Aflatoxin M1 (M+H) m/z 329/229 CE 41.7 V (RF lens 170 V) ; m/z 329/273.1 CE 24.5 V (RF lens 170 V)

Recoveries**	
Analyte	Aflatoxin M1
Standard*	100
0,01 ppb	101
0,025 ppb	92
0,05 ppb	96
0,1 ppb	93
0,1 ppb	91
0,1 ppb	90
0,25 ppb	90
0,5 ppb	86
0,75 ppb	93
1,0 ppb	87
1,5 ppb	92

* Standard was set = 100%
** Corrected with non-spiked sample / The results meet the performance criteria according to EC 406/2001, EC 519/2014 and the performance criteria for aflatoxin M1 recovery set by the AOAC. For the determination of robustness, multiple columns were used for each data point (n=3).
All statistical errors and deviations of the results of the experiments were below 4%.

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