

Determination of aflatoxin M1 in milk

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SUMMARY

This application describes a fast and isocratic method for the determination of aflatoxin M1 in milk and raw milk with an easy post column derivatization step using a UVE photochemical reactor. Furthermore, required sample preparation via solid phase extraction (SPE) is recommended.

INTRODUCTION

Aflatoxins are the best known group of mycotoxins that were named after the main fungi strain producing them as secondary metabolites namely *Aspergillus flavus*. Aflatoxins are also produced by *Aspergillus parasiticus* and to a smaller extent also by other strains. Aflatoxins can accumulate on crops in the field or during storage of agricultural products, especially under warm and humid conditions. Unfortunately, these substances can persist long after the fungi have been killed and therewith contaminate foods. The more common aflatoxins, which include G2, G1, B2, and B1, have been identified as contaminants in cattle feed. Upon ingestion, aflatoxins B2 and B1 are metabolized to M2 and M1, potentially adulterating dairy products. The maximum aflatoxin M1 level set by the U.S. Food and Drug Administration and European Commission is 0.5 µg/L. [1,2].

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RESULTS

First, the analytical method was developed using a standard solution. **Fig 1** shows the fluorescence chromatogram with post column derivatization using the UVE photochemical reactor for an aflatoxin M1 standard at a concentration of 1 $\mu\text{g/mL}$. To make sure that the legal limit value is detectable, a milk sample was spiked with aflatoxin M1 to a concentration of 0.5 $\mu\text{g/L}$ and pretreated with online solid phase extraction. **Fig 2** shows an overlay of the spiked milk sample after sample preparation and the aflatoxin M1 standard. Although matrix effects occur through SPE pretreatment it was possible to quantify aflatoxin M1 in the measured milk sample spiked down to 0.5 $\mu\text{g/L}$. For sample pretreatment following SPE procedure was conducted [4]: 20 mL of the spiked milk were diluted with 30 mL distilled water. A CHROMABOND® C18 ec SPE column was conditioned with 10 mL methanol and subsequently with 10 mL water. After this the sample was slowly forced or aspirated through the column. The SPE column was washed with 10 mL water and 10 mL n-hexane. Afterwards the column was dried for 10-20 min at 50°C or overnight at ambient temperature. After drying the sample was eluted with 3 mL acetonitrile.

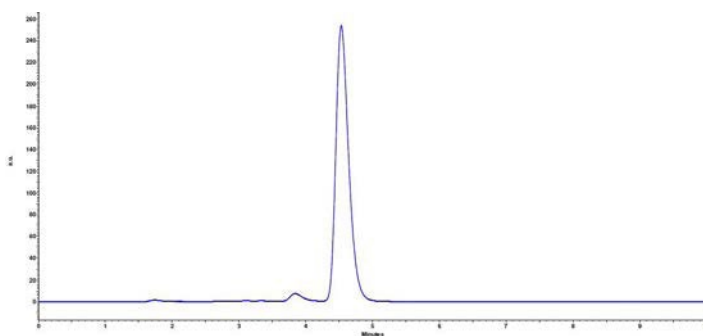


Fig. 1 Chromatogram aflatoxin M1 standard 1 $\mu\text{g/mL}$

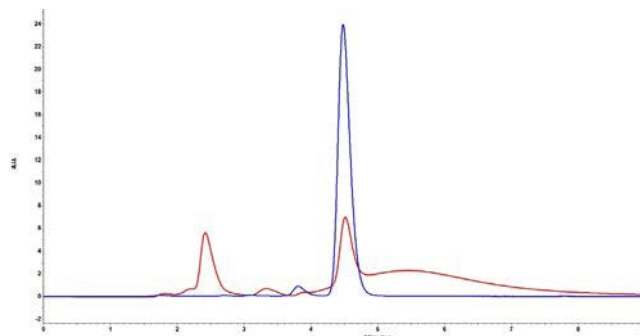


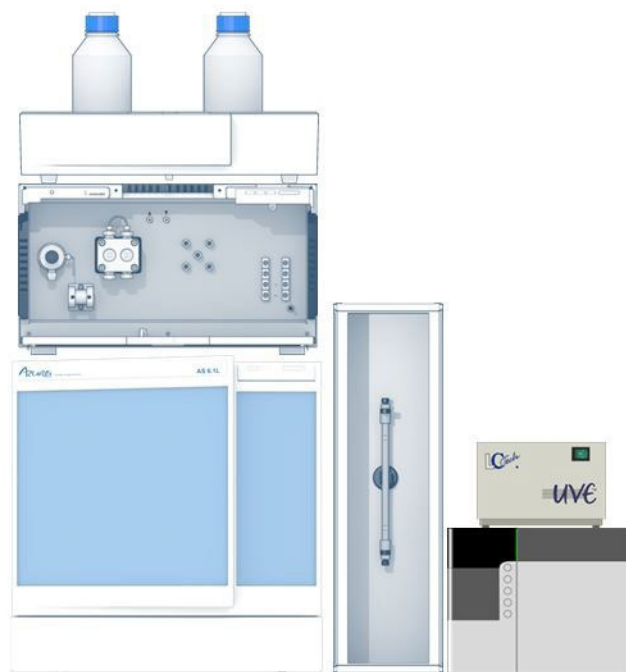
Fig. 2 Overlay of spiked milk sample after SPE (red) and standard (blue)

MATERIALS AND METHODS

An AZURA Analytical HPLC Plus system was used for this application. It consisted of an AZURA P 6.1L LPG pump, an autosampler 3950, an AZURA CT 2.1 column thermostat, the UVE photochemical reactor and fluorescence detector RF-20Axs. The analytical method was run isocratically at a flow rate of 0.8 mL/min with a mixture of water, methanol and acetonitrile 60:25:15 (v/v). The column thermostat was set to 30 °C and the detector was set to excitation 365 nm/emission 455 nm. The sensitivity was adjusted to high with a gain of 16. The used column was filled with KNAUER Eurospher II 100-3 C18 silica.

CONCLUSION

Using the UVE photochemical reactor for post column derivatization in combination with the AZURA Analytical HPLC system and fluorescence detection, the valid maximum limit values of 0.5 µg/L for aflatoxin M1 in milk and other dairy products could be quantified.



REFERENCES

- [1] FDA Mycotoxin Regulatory Guidance, National Grain and Feed Association 1250 Eye St., N.W., Suite 1003, Washington, D.C., 20005-3922 August 2011, <http://www.ngfa.org/wp-content/uploads/NGFAComplianceGuide-FDARegulatoryGuidance-forMycotoxins8-2011.pdf>
- [2] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union, L 364/5 - L 364/24, 20.12.2006, <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&qid=1487915647230&from=EN>
- [3] <http://www.mn-net.com/DesktopModules/TabID/10160/default.aspx>

ADDITIONAL RESULTS

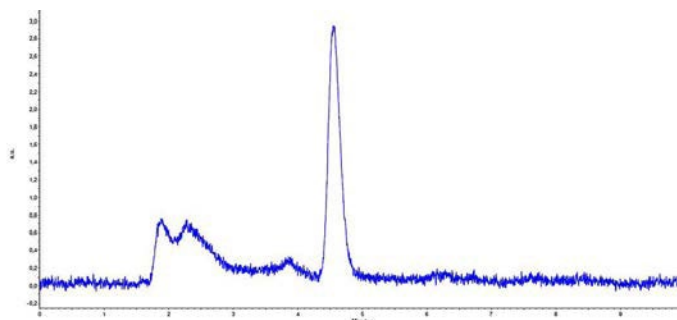


Fig. A1 Chromatogram aflatoxin M1 standard 0.001 µg/mL

ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Eluent A	Water/Methanol/Acetonitrile 60:25:15		
Gradient	Isocratic 100 % A		
Flow rate	0.8 mL/min	System pressure	260 bar
Column temperature	30 °C	Run time	10 min
Injection volume	10 µL	Injection mode	Full loop
Detection wavelength	Ex 365 nm/Em 455 nm	Data rate	5 Hz
		Time constant	0.2 s



Tab. A2 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, LPG 10mL, SSt	APH34EA
Autosampler	3950 analytical version	AYASM
Detector	RF-20Axs	A59201
Thermostat	AZURA CT 2.1	A05852
Software	OpenLAB CDS EZChrom Edition	A2600-1
Column	Vertex Plus Column, 150x3 mm ID with precolumn, Eurospher II 100-3 C18	15XE181E2G
Post column derivatisation	UVE photochemical reactor	A07547

RELATED KNAUER APPLICATIONS

VFD0146 – Sensitive online SPE determination of Bisphenol A in water samples

VFD0159 – Alternaria alternata – determination of main metabolites

VFD0158 – Zearalenone and its major metabolites – a simple isocratic method