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# DETERMINATION OF AFLATOXIN M1 IN MILK

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## SUMMARY

This application note 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].

## 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 µg/mL. To make sure that the legal limit value is detectable, a milk sample was spiked with aflatoxin M1 to a concentration of 0.5µg/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 µg/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.

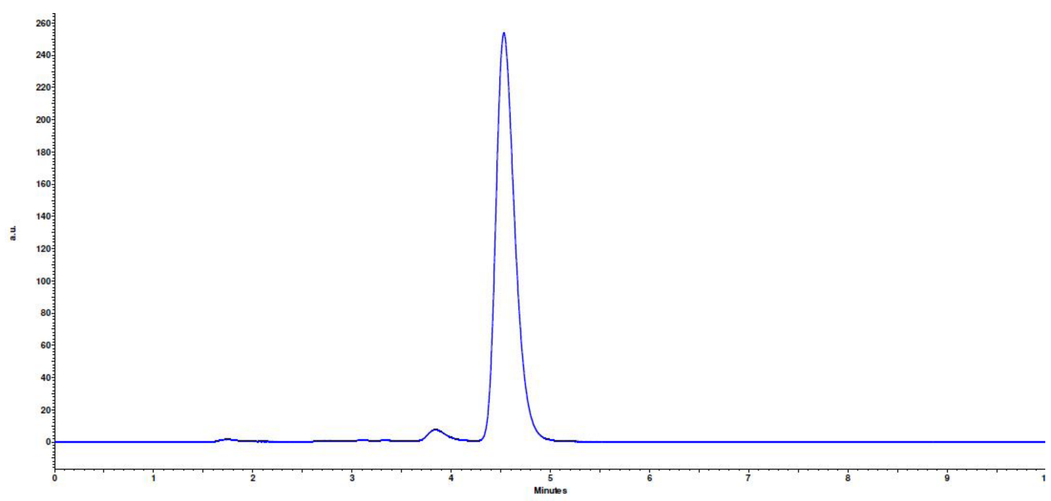


**Fig. 1** Chromatogram aflatoxin M1 standard 1 µg/mL

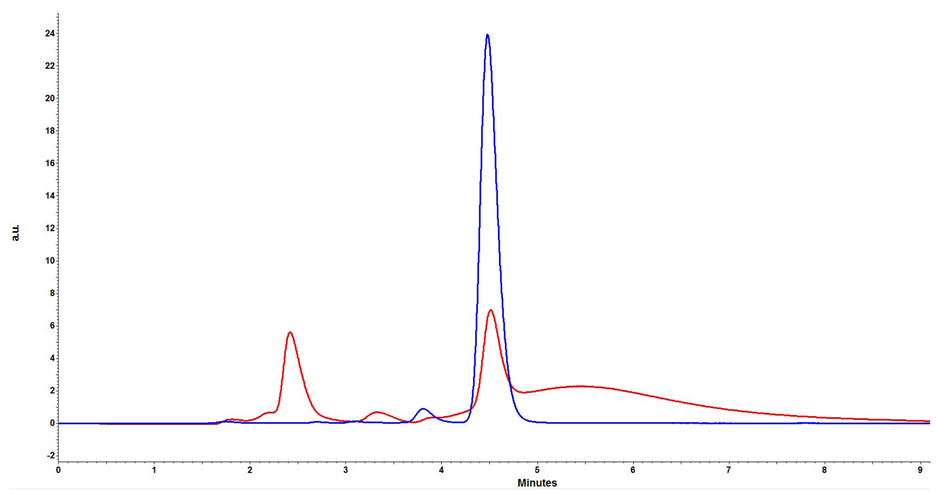


**Fig. 2** Overlay of spiked milk sample after SPE (red) and standard (blue)

## MATERIALS AND METHOD

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-FDARegulatoryGuidanceforMycotoxins8-2011.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/defauld.aspx

**Additional information:**

VFD0152

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## ADDITIONAL RESULTS

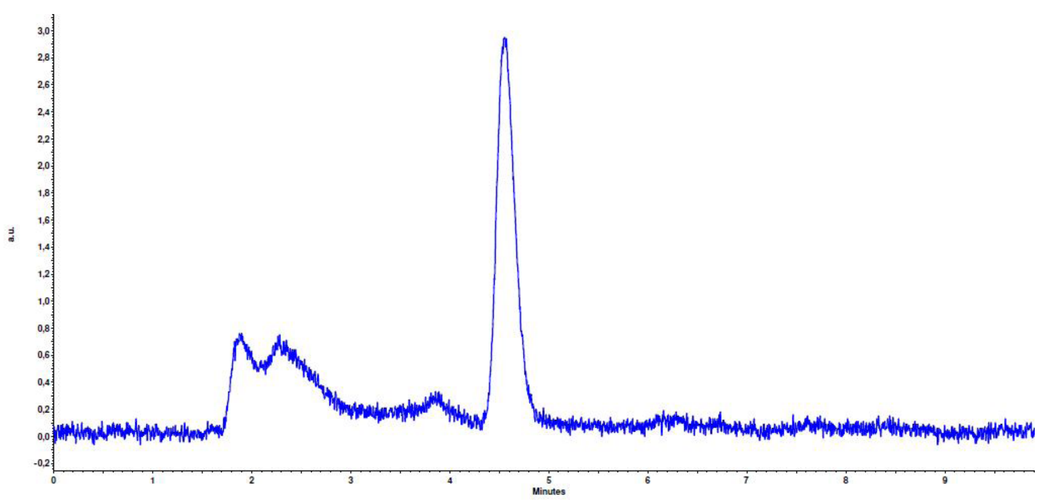
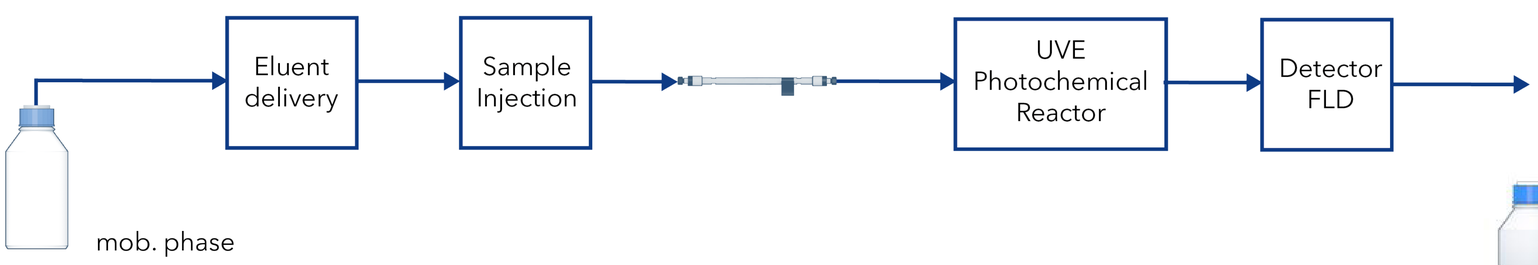



**Fig. A1** Chromatogram aflatoxin M1 standard 0.001 µg/mL

## 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 & data

| Instrument | Description | Article No. |
|---|---|---|
| Pump | AZURA P 6.1L, LPG 10mL, SSt | APH34EA |
| Autosampler | 3950 analytical version | A50070 |
| 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 |