Science Together

Quick and easy determination of **Aflatoxins** in food matrices

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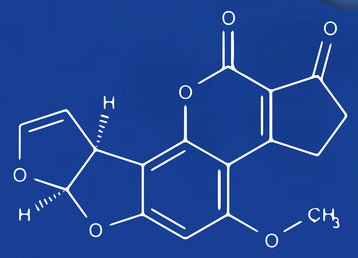
SUMMARY

Cultivation and storage of food and animal feed may contribute to the spread of molds, which produce mycotoxins – toxic secondary metabolites. Their consumption can lead to serious health damage in both, humans and animals. Therefore, it is inevitable to provide a simple and exact method for the analysis of mycotoxins in food, especially the most often occurring aflatoxins to ensure consumer safety. Here, a fast method for the determination of aflatoxins is described with an easy derivatization step using the UVE photochemical reactor.



INTRODUCTION

Aflatoxins are the best-known group of mycotoxins produced as secondary metabolites by fungi, mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, but to a smaller extent also by other strains. This origin is also where the name Aspergillus flavus toxin comes from. Since they can easily enter the marketplace and be hazardous to public health it is important to develop effective analytical methods for the identification and quantification of mycotoxins. The big challenge thereby is that already minimal amounts are toxic and need to be detected reliably. Aflatoxins occur in different food- and feedstuffs e.g. cereals, nuts, and milk products. Practically this means that analysis from very different sample matrices must be easy and fast (**Fig. 1**). In this case analysis is performed with an HPLC system that contains an UVE photochemical reactor located right after the column. With this reactor the so called photochemical derivatization of the non-visible Aflatoxins to their fluorescent derivates (**Fig. 2**) is realised. Here, we present not only an application for easy and fast determination of aflatoxins but a very affordable all-in-one system solution.

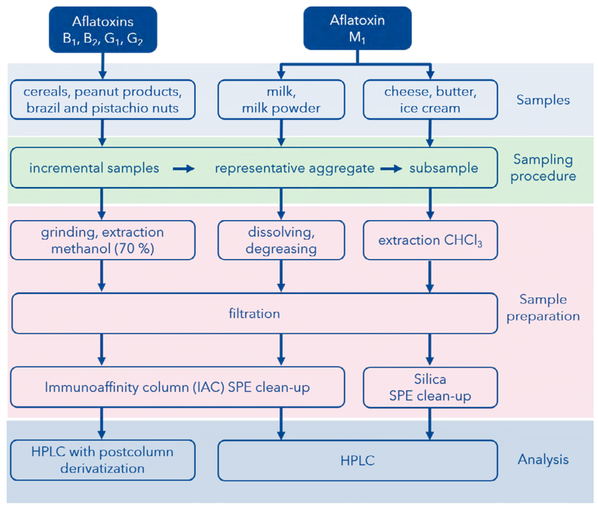


Fig. 1 General analysis procedure for aflatoxin determination

INFO BOX: POST-COLUMN DERIVATIZATION

Post-column derivatization alters the physical properties of molecules normally invisible to the given detection method. The derivatization is accomplished e.g. with UV radiation (photochemical) or addition of a functional group (chemical) which is applied to the separated sample. Commonly, the a derivatization method is chosen that produces a strong colour or a fluorescent product. The sensitivity of detection can be thereby significantly increased. It can be stated that generally post-column derivatization is an easy method to increase the selectivity and sensitivity of HPLC analyses.

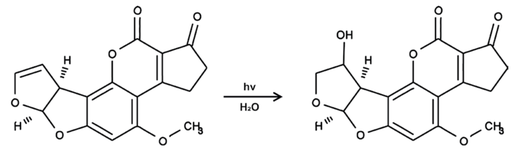


Fig. 2 Photochemical derivatization of Aflatoxin B_1 to B_{2a}

Additional information

RESULTS

Here, the separation of four typical aflatoxins from nut and cereal samples is demonstrated. The detection took place with the help of post-column derivatization using an UVE photoreactor.

As shown in **Fig. 3** the peaks were separated well and showed a decent shape. Also, the run time was short enabling fast analyses.
Moreover, it was shown that the photochemical derivatization (green graph) lead to higher and sharper peaks for Aflatoxins G_1 and B_1 than with-out (blue graph).
It has been shown before that this method is also suitable for the determination of Aflatoxin M_1 from milk (see AppNote VFD0152).

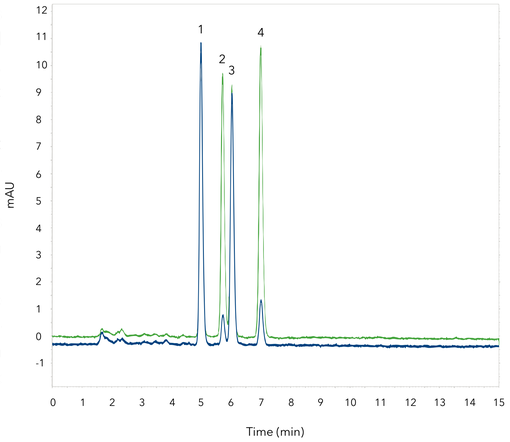
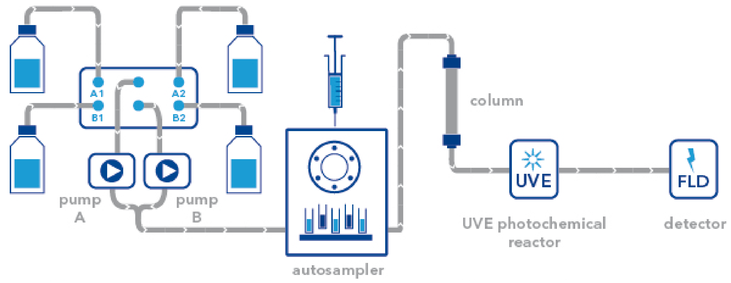


Fig. 3 Comparison of FLD chromatogrammes for the aflatoxin analysis with (green) and without photochemical post-column derivatization (blue) of standard mix containing 1) Aflatoxin G_2, 2) Aflatoxin G_1, 3) Aflatoxin B_2, and 4) Aflatoxin B_1

MATERIALS AND METHODS

Nut and cereal samples were first homogenized in a methanol/water mixture and filtered. The filtrate was dissolved in an aqueous solution and degreased with hexane. After extraction with chloroform and subsequent concentration, the extract was applied to an SPE cartridge. The elution step was done with chloroform/acetone so that the sample is afterwards ready for injection into the HPLC system. The analysis is done via a classical HPLC method on a C18 column (Eurospher II 100-3 C18, 250 x 3 mm ID) using a gradient method with water and acetonitrile as eluent. Detection is done with a fluorescence detector (ex 365 nm/em 455 nm) and post-column derivatization with the UVE photoreactor to lower the detection limit of aflatoxin B_1 and G_1.



CONCLUSION

Using the UVE photoreactor for post-column derivatization in combination with an HPLC method on the AZURA Analytical system and a Eurospher II C18 column, it was easily possible to analyze all four aflatoxins. The mobile phase is not altered for this post-column derivatization method, so the analysis of other substances is not affected. Since the peaks were well separated with high resolution it would also be possible to analyze even more mycotoxins with this method. As a conclusion, it can be stated that UVE is highly recommendable for the fast and easy analysis of mycotoxins from food matrices thereby not having to rely on expensive equipment like MS.