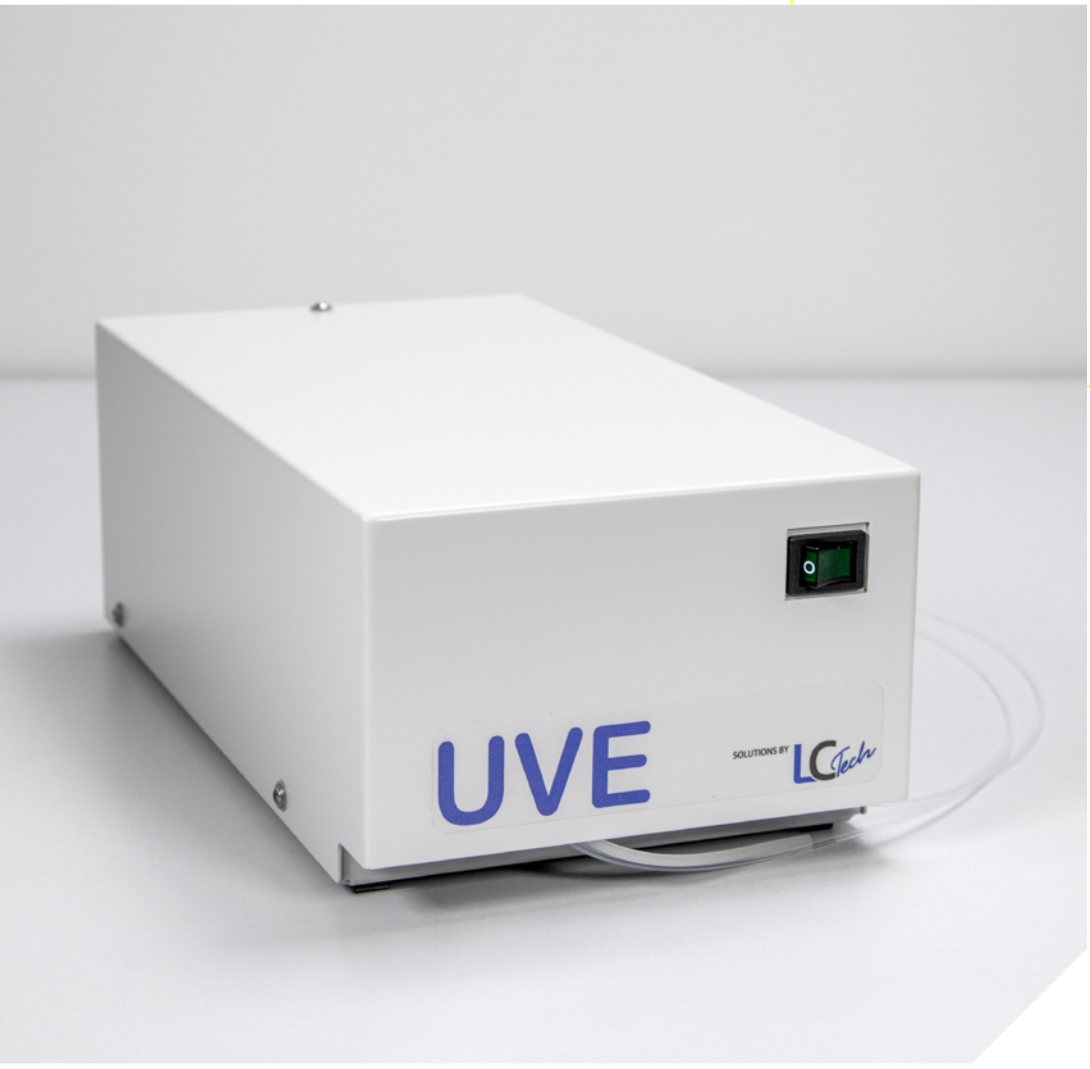
# Aflatoxin-HPLC Analysis

## with photochemical post column derivatization

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# Aflatoxin-Analysis

Aflatoxins are naturally occuring toxins (mycotoxins = fungal toxins), which are highly toxic for humans and mammals. The aflatoxins are produced by molds (e.g. Aspergillus flavus). They are highly cancercausing, especially in the liver.

Aflatoxins are found in nuts, figs, dates, com, dried paprika, spices and many other vegetable foodstuff.

The abbrevations of Aflatoxins are made up of the color of the fluorescence (blue or green) or the occurance and its relative chromatographic mobility (1 or 2).

Aflatoxin M1 (M = Milk) results from Aflatoxin B1 after absorption of feed in the animal organism and can be found again in the milk.

Because of their harmful effects on human beings, the acceptable maximum value in numerous matrices are controlled in the regulation EG (European Community) No. 1881/2006.

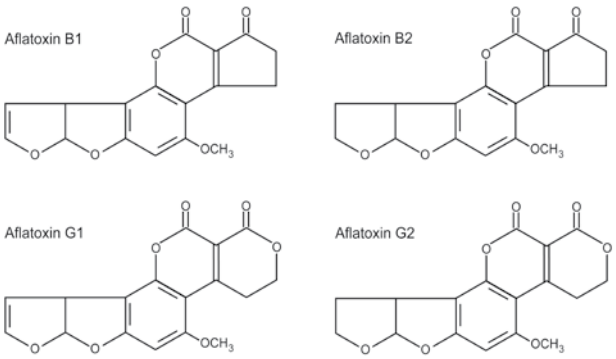



Fig. 1: Structure of Afla-

# Method description

To measure aflatoxins via HPLC and fluorescence detection it is often necessary to include post column derivatization. Otherwise partly very low maximum values for aflatoxins in food cannot be reached due to low fluorescence of the most important aflatoxins B1 and G1.

The sensitivity of the measurement is significantly enhanced by derivatization of aflatoxins B1 and G1. In combination with modern HPLC devices resp. fluorescence detectors further labor-intensive steps like concentration to dryness and subsequent solution in the HPLC eluent often can be left out. Moreover matrix loadings in general can be lower, advantageous for the entire working process.

According to matrix requirements, aflatoxins are extracted with organic solvents like methanol or acetonitrile. Subsequently the diluted raw extract is cleaned up with immunoaffinity columns followed by HPLC, derivatization and fluorescence detection at 365/460 nm. The separation is done with a special analytical 150 mm column with guard column. Running time is short at nevertheless excellent baseline separation.

Attainable detection limits are in the low ppt area according to system and sample preparation.

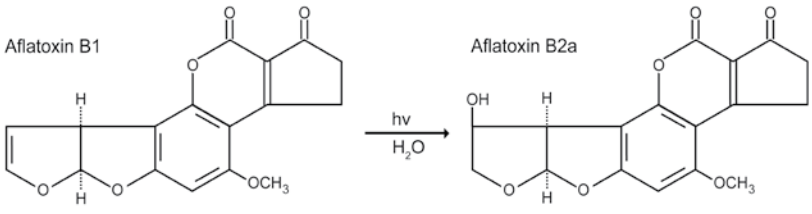



Fig. 2: Photochemical derivatization of Aflatoxin B1 to B2a

Fig. 3: 3 mL AflaCLEAN immunoaffinity columns

# UVE - Photochemical Post Column Derivatization

As described above, only the aflatoxins B2 and G2 show own fluorescence. Thus, B1 and G1 have to be derivatized for suitable detection. This can be done easily, comfortably or at low costs with the UVE (photochemical radiation with UV light at 254 nm).

The UVE simply is connected with the column exit of the HPLC device and the entry of the fluorescence detector. No reagents are needed, because the water in the HPLC eluent is used for it. Another advantage is the direct confirmation analysis by simply switching off the device.

This method is equal to the established derivatization using bromine, as shown in some publications (e.g. A. Papadopoulou-Bouraoui, J. Stroka, E. Anklam, J. AOAC Intl. 85, No. 2, 2002, 411 – 416). The system is used world-wide in accredited public and private laboratories and has shown excellent performance in FAPAS proficiency tests 0490/2006 „Aflatoxin analysis in pistachios", 04143/2009 „Aflatoxin Analysis in Baby Food" and 04148/2009 „Aflatoxins B&G in Maize".

During photochemical reaction by the UVE the aflatoxins B1 and G1 are hydroxilized (adsorption to a double bound), resulting in a stable measurable fluorescence. The chemical properties of the other aflatoxins (B2, G2), which are important for measurement, are not changed during this photochemical reaction.

The detection limit is mainly influenced by sample preparation and in particular by the performance of the detector. If the detector shows sufficient sensitivity, the alfatoxins can be measured directly after elution with AflaCLEAN respectively Afla-OtaCLEAN column without further steps.

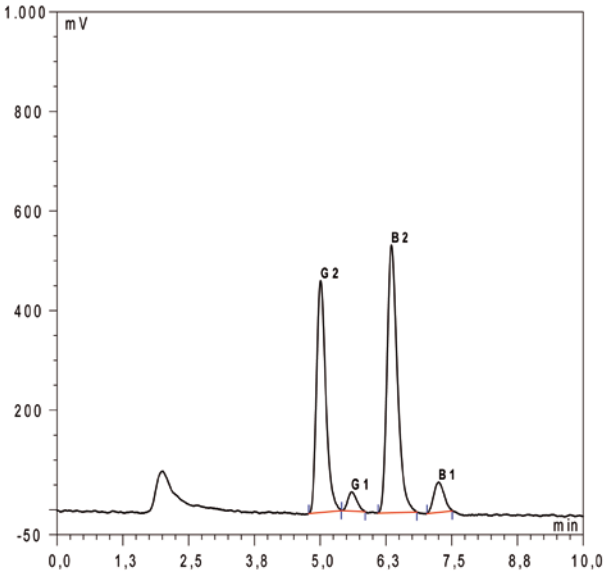

Abb. 4: UVE, module for photochemical derivatization.

## UVE, Technical Data

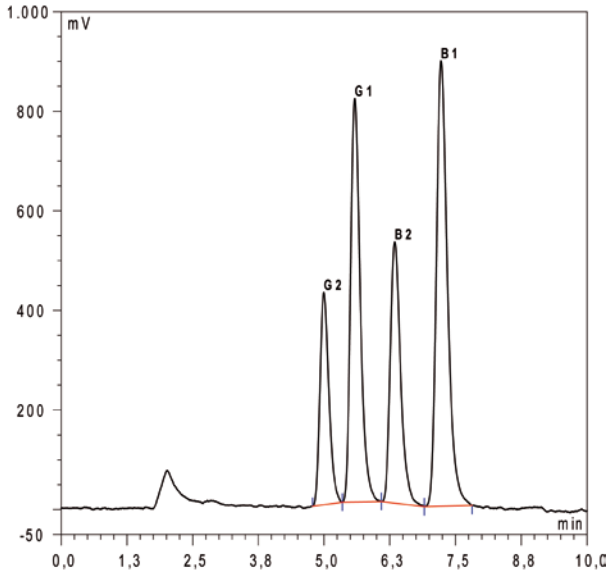
| | |
|---|---|
| Power supply | 220 – 265 V, 50/60 Hz |
| Power input | 100 W |
| Dimensions (w x h x d) | 145 × 100 × 275 mm |
| Weight | 3 kg |
| Reactor loop | 1 mL |

UVE is a registered trademark of LCTech GmbH, Obertaufkirchen, Germany

## Chromatograms




5a: Without photochemical derivatization (UVE switched off)




5b: After photochemical derivatization at 254 nm with the UVE

Abb. 5: Chromatograms of the aflatoxins G2 (5.0 min), G1 (5.8 min), B2 (6.6 min) and B1 (7.7 min); measured on LCTech special column with guard column for aflatoxin analysis

Enhancement of fluorescence is at about 30 times for the aflatoxins B1 resp. G1 Injected were 0.25 ng (B2, G2) and 1 ng (B1, G1) in each chromatograms. Peak width of Aflatoxin B1 at half peak height in 5a is 13.6 s.

| HPLC | |
|---|---|
| Operation mode | Isocratic |
| Eluent | Water/acetonitrile/methanol (60/15/30 // v/v/v) |
| HPLC column | 150 × 4.6 mm; C-18 with guard column |
| Column temperature | 36 °C |
| Flow rate | 1.2 mL/min |
| Injection volume | 10 – 100 µL |

| Post Column Derivatization | |
|---|---|
| Photochemical reactor UVE | 254 nm |
| Reactor volume | 1 mL |

| Detection | |
|---|---|
| Measure mode | Fluorescence detection |
| Wave length for excitation | 365 nm |
| Wave length for emission | 460 nm |

## Literature

1) A. Papadopoulou-Bouraoui, J. Stroka, E. Anklam, J. AOAC Intl. 85, No. 2, 2002, 411 – 416

2) V.S. Sobolev, J.W. Dorner, J. AOAC Intl. 85, No. 3, 2002, 642 – 645

3) AOAC Official Method 2005.08, Aflatoxins in Corn, Raw Peanuts, and Peanut Butter; Liquid Chromatography with Post-Column Photochemical Derivatization

| Ordering Information | |
|---|---|
| 10519 | Photochemical reactor UVE, 1 mL reactor volume |
| 10563 | UVC lamp for photochemical reactor UVE |
| 10520 | Reactor coil for photochemical reactor, 1 mL |
| 10514 | AflaCLEAN, immunoaffinity columns, 3mL PP wide bore, 25/pck |
| 11022 | Afla-OtaCLEAN, immunoaffinity columns, 3mL PP wide bore, 25/pck |
| 10522 | HPLC column for aflatoxin analysis, 150 × 4.6 mm, C18 |
| 10750 | Guard cartridge holder with 3 cartridges 10523 for 10522 |
| 10523 | Guard cartridges for 10522, 3/pck |

# Any Questions?
# Do not hesitate to contact us:

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