



# HPLC Analysis of Aflatoxines in Pistachio Nuts using HPLC

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Food

## Abstract

The following mycotoxins have been analyzed: aflatoxins G<sub>2</sub>, G<sub>1</sub>, B<sub>2</sub>, B<sub>1</sub>, M<sub>2</sub>, and M<sub>1</sub>, ochratoxin A, zearalenone, and patuline.

Mycotoxins are highly toxic compounds produced by fungi. They can contaminate food products when storage conditions are favorable to fungal growth. These toxins are of relatively high molecular weight and contain one or more oxygenated alicyclic rings. The analysis of individual mycotoxins and their metabolites is difficult because more than 100 such compounds are known, and any individual toxin is likely to be present in minute concentration in a highly complex organic matrix. Most mycotoxins are assayed with thin-layer chromatography (TLC). However, the higher separation power and shorter analysis time of HPLC has resulted in the increased use of this method. The required detection in the low parts per billion (ppb) range 4,<sup>1,2,3</sup> can be performed using suitable sample enrichment and sensitive detection.

## Sample preparation

Samples were prepared according to official methods.<sup>2</sup> Different sample preparation and HPLC separation conditions must be used for the different classes of compounds. The table on the next page gives an overview of the conditions for the analysis of mycotoxins in foodstuffs.

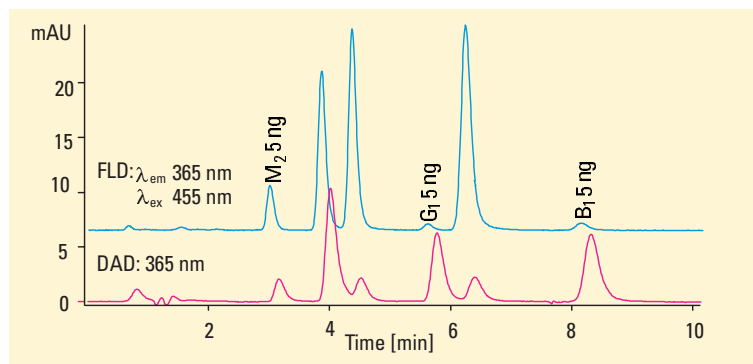


Figure 1  
Analysis of aflatoxins with UV and fluorescence detection

## Chromatographic conditions

The HPLC method presented here for the analysis of mycotoxins in nuts, spices, animal feed, milk, cereals, flour, figs, and apples is based on reversed-phase chromatography, multisignal UV-visible diode-array detection, and fluorescence detection. UV spectra were evaluated as an additional identification tool.



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## HPLC method performance

Limit of detection 1–5 µg/kg

Repeatability  
of RT over 10 runs <0.12 %  
of areas over 10 runs <1.5 %

Linearity  
of UV-visible DAD 1–500 ng  
of fluorescence 30 pg to 2 ng

Column class	Matrix	Sample preparation	Chromatographic conditions
Aflatoxins G <sub>2</sub> , G <sub>1</sub> , B <sub>2</sub> , B <sub>1</sub> , M <sub>2</sub> , M <sub>1</sub>	nuts, spices, animal feed, milk, dairy products	• extraction according to Para. 35, LMBG* 4, 5	Hypersil ODS, 100 ~ 2.1 mm id, 3-µm particles water/methanol/ACN (63:26:11) as isocratic mixture (100% B is recommended for cleaning the column flow rate: 0.3 ml/min at 25 °C DAD: 365/20 nm Fluorescence detector (FLD): excitation wavelength 365 nm, emission wavelength 455 nm
Ochratoxin A	cereals, flour, figs	• extraction according to Para. 35, LMBG • acidify with HCl • extract with toluene • SiO <sub>2</sub> cleanup elute toluene/acetic acid (9:1)	Lichrospher 100 RP18, 125 ~ 4 mm id, 5-µm particles water with 2 % acetic acid/ACN (1:1)* flow rate: 1 ml/min at 40 °C FLD: excitation wavelength 347 nm, emission wavelength 480 nm
Zearalenone	cereals	• extract with toluene • Sep-pak cleanup • elute toluene/acetone (95:5) • AOAC 985.18: <sup>3</sup> α-zearalenol and zearalenone in corn	Hypersil ODS, 100 ~ 2.1 mm id, 3-µm particles water/methanol/ACN (5:4:1) as isocratic mixture* flow rate: 0.45 ml/min at 45 °C DAD: 236/20 nm FLD: excitation wavelength 236 nm, emission wavelength 464 nm
Patuline	apple products	• cleanup on Extrelut • silica gel cleanup • elute toluene/ ethylacetate (3:1)	Superspher RP18, 125 ~ 4 mm id, 4-µm particles water 5 %–95 % ACN flow rate: 0.6 ml/min at 40 °C DAD: 270/20 nm or Lichrospher diol, 125 ~ 4 mm id, 5-µm particles hexane/isopropanol (95:5) as isocratic mixture flow rate: 0.6 ml/min at 30 °C DAD: 270/20 nm

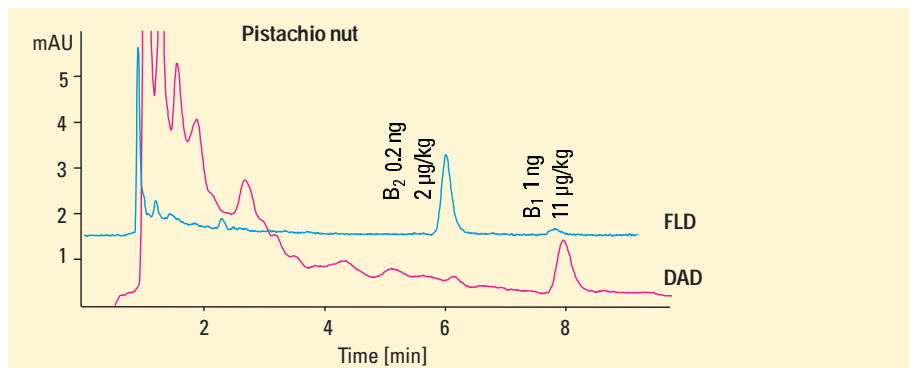


Figure 2  
Analysis of aflatoxins in pistachio nuts with UV and fluorescence detection

## References

1. R. Schuster, G. Marx, G. M. Rothaupt, "Analysis of mycotoxins by HPLC with automated confirmation by spectral library", *Agilent Application Note* 5091-8692, 1993.
2. Lebensmittel- und Bedarfsgegenstandesgesetz, Paragraph 35, Germany.
3. *Official Methods of Analysis, Food Compositions; Additives, Natural Contaminants*, 15th ed; AOAC: Arlington, VA, 1990, Vol. 2.; AOAC Official Method 980.20: aflatoxins in cotton seed products; AOAC Official Method 986.16: Aflatoxins M<sub>1</sub>, M<sub>2</sub> in fluid milk; AOAC Official Method 985.18: α-zearalenol.
4. Farrington et. al., "Food Additives and Contaminants, 1991, Vol. 8, No. 1, 55-64".
5. *Official Methods of Analysis*, Horwitz, W., Ed.; 14th ed.; AOAC: Arlington, VA, 1984; secs 12.018–12.021.

## Equipment

### Agilent 1100 Series

- degasser
  - isocratic pump
  - autosampler
  - thermostatted column compartment
  - diode array detector,
  - fluorescence detector
- Agilent ChemStation  
+ software

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