Application Note

Determination of mycotoxins with photochemical post column derivatization

Category	Food
Matrix	Food and feedstuff
Method	HPLC
Keywords	Aflatoxins, mycotoxins, food analysis, food safety, photochemical post column derivatization
Analytes	Aflatoxin (G2, G1, B2, B1), Ochratoxin A Zearalenone, Deoxynivalenol
ID	**VFD0141N_A_E, 07/16**

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. Therewith, 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. In this application note a fast method for the simultaneous determination of aflatoxins and other relevant mycotoxins in one run is described with an easy derivatization step using the UVE photochemical reactor.

Introduction

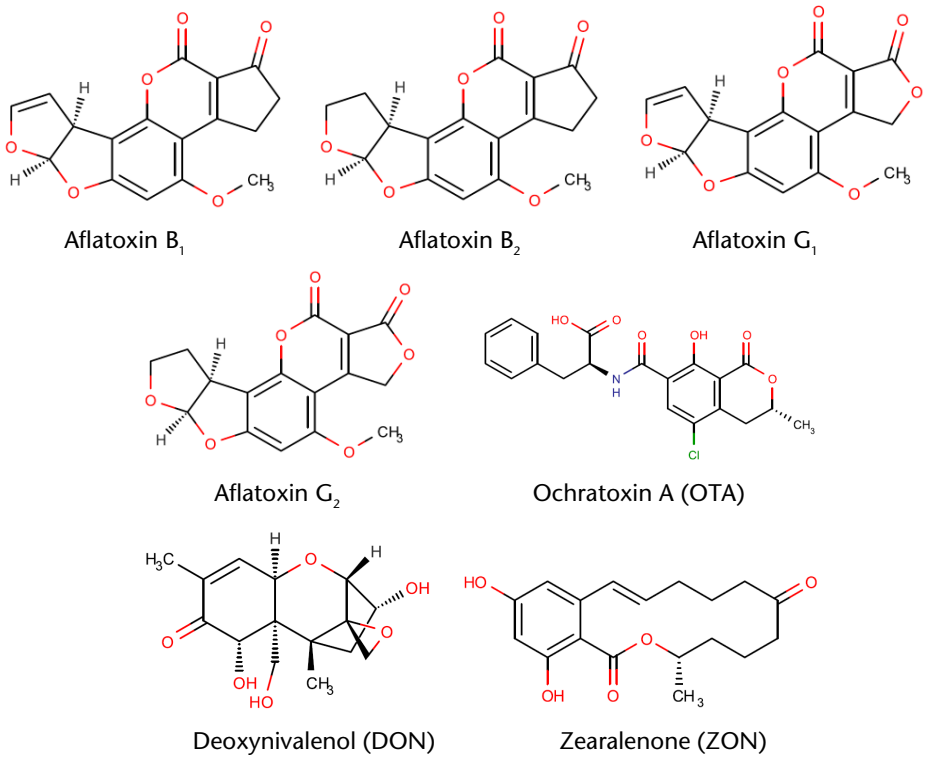
Aflatoxins are the best known group of mycotoxins produced as secondary metabolites by fungi, mainly by *Aspergillus flavus* and *Aspergillus parasiticus,* but to a smaller extent also by other strains. This origin is also where the name ***A**spergillus **fla**vus **toxin*** comes from. Aflatoxins can be produced on crops in the field or during storage of agricultural products, especially under warm conditions and high humidity. Unfortunately, these substances can persist long after the fungi have been killed and therewith contaminate foods. Most mycotoxins are stable compounds that are also not destroyed during food processing or cooking. Although a large number of aflatoxins exist, only a limited number is important in (analytical) practice.

Aflatoxin B_1 is most widespread and can be found in food and feed products such as peanuts, corn and cottonseed. It is highly toxic and the WHO classified it as a group 1 carcinogen. The aflatoxins B_2, G_1, and G_2, are usually found accompanying B_1, in lower concentrations in the contaminated samples. The order of toxicity is AFB1 > AFG1 > AFB2 > AFG2.[1] Ochratoxin A (OTA) is a mycotoxin which is formed from different species of Penicillium and Aspergillus. Its generation can take place during plant growth but frequently it is generated in the course of storage and converting of food.[4] Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect corn, wheat, oats, barley, rice, and other grains in the field or during storage. The exposure risk to humans is directly through foods of plant origin (cereal grains) or indirectly through food of animal origin (kidney, liver, milk, eggs). It has been detected in buckwheat, sorghum, triticale and other food products including flour, bread, breakfast cereals, popcorn, noodles, infant foods, pancakes, malt and beer. DON affects animal and human health causing acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever.[3] Zearalenone (ZON) is a mycotoxin belonging to the genus Fusarium and is produced mainly in food and feed. It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperoestrogenic syndromes in humans.[4]

Since mycotoxins can easily enter the market and be a hazard to public health it is important to develop effective analytical methods for their identification and quantification. Governmental institutions and health protection agencies apply these methods on a large scale to control marketed food products and animal feed. In the food processing industry the same methods are used to check raw materials and products, in order to direct them to countries with an appropriate legislation.[5]

Fig. 1

Chemical structure of the determined aflatoxins



Additionally, the presence of aflatoxins B1, B2, G1, and G2 in a variety of processed and unprocessed foods is controlled in countries around the world. Charts 1 shows exemplarily action limits set by the U.S. Food and Drug Administration (levels where the FDA will take legal action to remove products from the market). Charts 2 to 5 show limits for OTA, DON, ZON and maximum aflatoxin levels set by the European Commission according to regulation EG 1881/2006 [5,6].

Chart 1

FDA action levels for aflatoxin in human food, animal feed and animal feed ingredients[5]

Intended use	Grain, grain by-product, feed or other products	Aflatoxin level [µg/kg]
Human consumption	Milk	0.5
Human consumption	Foods, peanuts and peanut products, brazil and pistachio nuts	20
Immature animals	Corn, peanut products and other animal feeds and ingredients, excluding cottonseed meal	20
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cottonseed, and other animal feeds and ingredients	20
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200
Finishing (i.e., feedlot) beef cattle	Corn and peanut products	300
Beef, cattle, swine or poultry, regardless of age or breeding status	Cottonseed meal	300

Chart 2

Maximum levels for DON in foodstuffs set by The European Commission[6]

Foodstuff	Maximum level [µg/kg]
Unprocessed cereals other than durum wheat, oats and maize	1250
Unprocessed durum wheat and oats	1750
Unprocessed maize	1750
Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits, bran as end product marketed for direct human consumption and germ	750
Pasta (dry)	750
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500
Processed cereal-based foods and baby foods for infants and young children	200

Chart 3

Maximum levels for certain contaminants in foodstuffs set by The European Commission[6]

Foodstuff	Maximum Aflatoxin Level [µg/kg] B1	Sum of B1, B2, G1, G2
Groundnuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	8.0	15.0
Nuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	5.0	10.0
Groundnuts and nuts and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
Dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	5.0	10.0
Dried fruit and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
All cereals and all products derived from cereals, including processed cereal products	2.0	4.0
Maize to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5.0	10.0
Raw milk, heat-treated milk and milk for the manufacture of milk-based products	-	-
Following species of spices: *Capsicum* spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika) *Piper* spp. (fruits thereof, including white and black pepper), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Curcuma longa* (turmeric)	5.0	10.0
Processed cereal-based foods and baby foods for infants and young children	0.10	-
Infant formulae and follow-on formulae, including infant milk and follow-on milk	-	-
Dietary foods for special medical purpose intended specifically for infants	0.10	-

Chart 4

Maximum levels for OTA in foodstuffs set by The European Commission[6]

Foodstuff	Maximum Level [µg/kg]
Unprocessed cereals	5.0
All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption	3.0
Dried vine fruit (currants, raisins and sultanas)	10.0
Roasted coffee beans and ground roasted coffee, excluding soluble coffee	5.0
Soluble coffee (instant coffee)	10.0
Wine (including sparkling wine, excluding liqueur wine and wine with an alcoholic strength of not less than 15 % vol) and fruit wine	2.0
Aromatized wine, aromatized wine-based drinks and aromatized wine-product cocktails	2.0
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape must as reconstituted, intended for direct human consumption	2.0
Processed cereal-based foods and baby foods for infants and young children	0.50
Dietary foods for special medical purposes intended specifically for infants	0.50
Green coffee, dried fruit other than dried vine fruit, beer, cocoa and cocoa products, liqueur wines, meat products, spices and liquorice	-

Chart 5

Maximum levels for ZON in foodstuffs set by The European Commission[6]

Foodstuff	Maximum level [µg/kg]
Unprocessed cereals other than maize	100
Unprocessed maize	200
Cereals intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption and germ	75
Maize intended for direct human consumption, maize flour, maize meal, maize grits, maize germ and refined maize oil	200
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize based breakfast cereals	50
Maize snacks and maize based breakfast cereals	50
Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children	20
Processed maize-based foods for infants and young children	20

Theoretical:
Recommended sample preparation

The traditional method for aflatoxin analysis includes additional to the sampling and grinding procedure an extraction step, sample clean-up using solid-phase extraction (SPE) via high-performance liquid chromatography (HPLC). Because of the time-consuming extraction and clean-up steps, sample throughput is limited using this technique.
Figure 2 gives an overview over the general steps used in aflatoxin analysis.

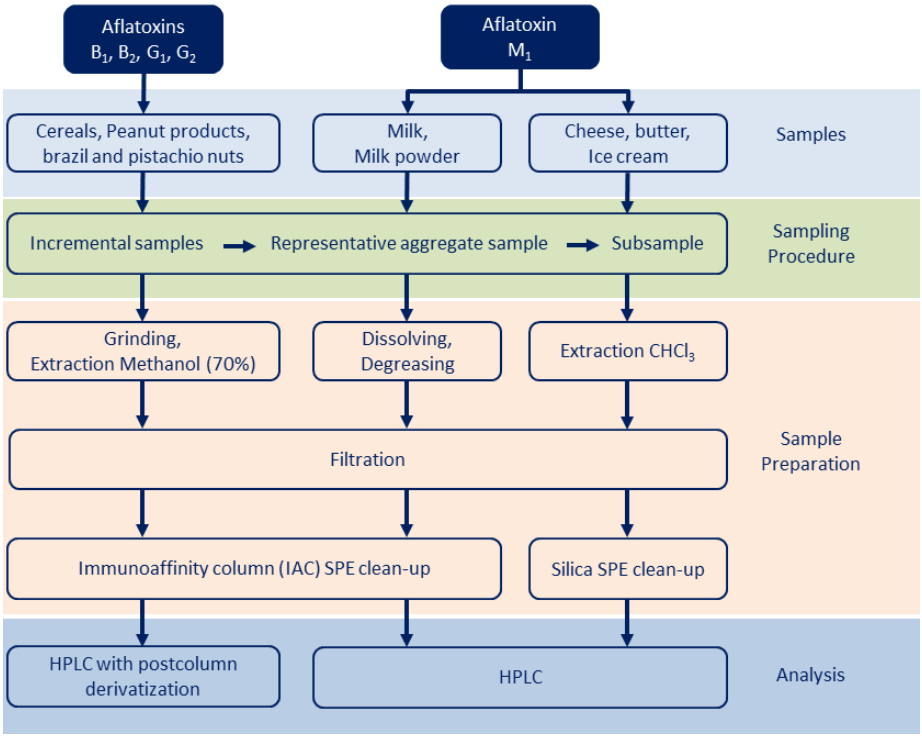


Figure 2
General analysis procedure for aflatoxin determination

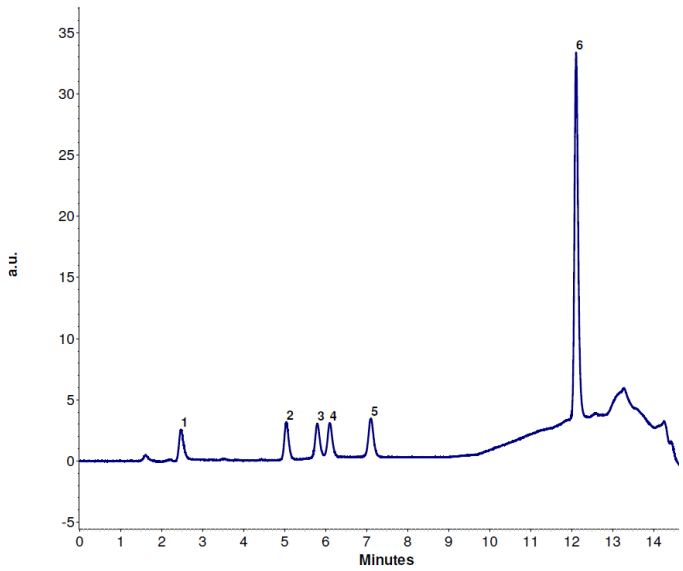
Method parameters

Aflatoxins B_1 and G_1 show only minimal fluorescence and are thus difficult to detect. Irradiating the aflatoxin mixture with UV light of 254 nm, the aflatoxins B_1 and G_1 undergo photo-induced hydroxylation and can then be measured through fluorescence spectrometry more sensitively.

Column	Eurospher II 100-3 C18, 250 x 4 mm ID with precolumn		
Eluent A	Water		
Eluent B	Acetonitrile		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	60	40
	6.00	60	40
	6.50	20	80
	10.00	10	90
	11.00	10	90
Flow rate	1.5 ml/min		
Injection volume	5 µl		
Column temperature	40 °C		
UV detection	220 nm (20 Hz)		
FLD detection	Ex 329 nm Em 460 nm (5 Hz, 0.1 s, Sensitivity: High, Gain 16) Post column derivatization with UVE photochemical reactor at 100 µA		

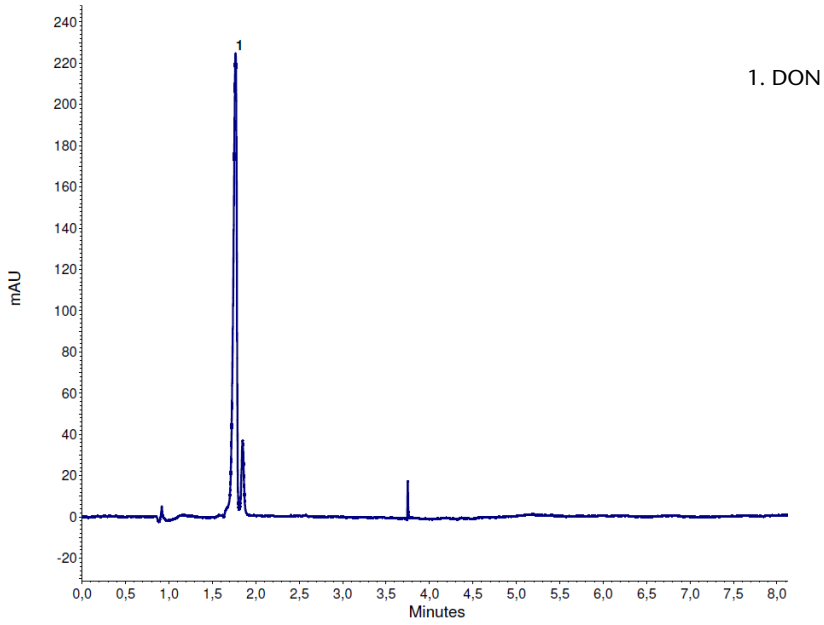
Results

Figure 1 shows the FLD chromatogram with post column derivatization using the UVE reactor. Figure 2 shows the DAD chromatogram for the same run where DON is detected. The separation works well on Eurospher II 100-3 C18 column. Peaks are baseline separated and show a good shape.



1. OTA
2. Aflatoxin G2
3. Aflatoxin G1
4. Aflatoxin B2
5. Aflatoxin B1
6. ZON

Fig. 1
FLD chromatogram for the mycotoxin analysis using UVE photochemical reactor for post column derivatization



1. DON

Fig. 2
DAD chromatogram at 220 nm for the mycotoxin analysis using UVE photochemical reactor for post column derivatization

Conclusion

Using the UVE photochemical reactor for post column derivatization in combination with the AZURA Analytical HPLC system and Eurospher II C18 column, it was possible to detect ZON, DON and the 4 aflatoxins B_1, B_2, G_1 and G_2 in one chromatographic run. The determination of OTA needs to be further elucidated. The mobile phase is not altered for this post column derivatization method, so the analysis of other substances is not affected. Also, the handling of the photochemical reactor was very easy because no further chemicals were required for derivatization. This application is an easy alternative for the determination of mycotoxins.

References

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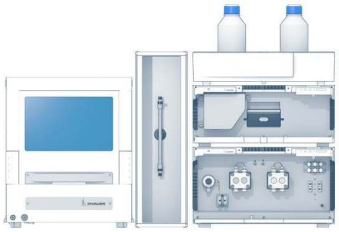
Physical properties of recommended column

Stationary phase	Eurospher II 100-3 C18
USP code	L 1
Pore size	100 Å
Pore volume	0.8 ml/g
Specific surface	320 ± 20 m^2/g
Particle size	3 µm
Form	spherical
% C	16
Endcapping	yes
Dimensions	250 x 4 mm ID with precolumn
Order number	25WE181E2G

Recommended instrumentation



Description	Order number
P 6.1L, binary HPG pump, 5 ml pump head	APH35GA
DAD 6.1L	ADC11
CT 2.1	A05852
AS 3950	A50070
Fluorescence Detector RF-20 Axs	A59201
LCTech UVE photochemical reactor	A07547

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