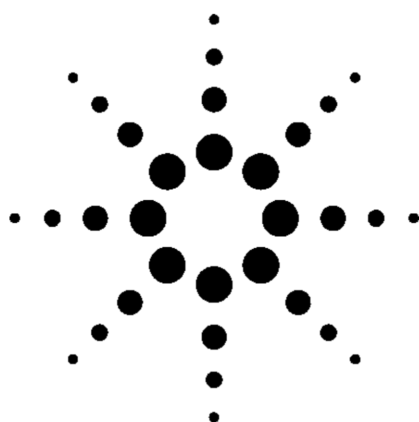
Separation of Aflatoxins by HPLC

Application



Environmental, Food Safety

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Abstract

Four target aflatoxins (B_1, B_2, G_1, and G_2) were separated by HPLC using an isocratic ternary mixture of water, methanol and acetonitrile, and detected using UV 365 nm. A baseline separation was achieved in less than 5.5 min.

Introduction

Aflatoxins are mycotoxins that are produced by various *Aspergillus flavus* molds. Not only are these compounds extremely toxic, but they are also mutagenic, teratogenic (causing fetal abnormalities), and carcinogenic. Unfortunately, *A. flavus* is a common mold found in tropical and subtropical countries and has been found to cause aflatoxin contamination. This contamination is a result of inadequate storage conditions for certain agricultural commodities such as peanuts, cereal seeds, dried fruit, and a wide range of tree nuts such as pistachio, pecans, walnuts, almonds, and herbal seeds such as red and black pepper, cloves, and cinnamon. Because of their toxic and carcinogenic nature, there is a very low minimum detectable quantity (MDQ) for aflatoxin contamination in food.

Chemical Nature

Although 18 different aflatoxins have been identified, the four most prevalent aflatoxins are B_1, B_2, G_1, and G_2, whose chemical structures are shown in Figure 1. Aflatoxin B_1 is one of the most potent and abundant environmental mutagens and carcinogens known. Aflatoxins are quite stable in many foods and are fairly resistant to degradation. Collectively, the aflatoxins are chemical derivatives of difurancoumarin. Pure aflatoxin B_1 is a pale-white to yellow crystalline, odorless solid. Aflatoxins are freely soluble in moderately polar solvents such as chloroform, methanol, and dimethyl sulfoxide, and dissolve in water to the extent of 10–20 mg/L. In methanol, they have fairly strong extinction coefficients (around 10,000) at 265 nm and 360–362 nm. They fluoresce under UV radiation, and fluorescence detection is often used for trace analysis in HPLC. Since there are differences in fluorescence yields between B_1 and B_2, and between G_1 and G_2, it can be useful to run both UV and fluorescence detectors in series [1]. Aflatoxins have no polar functional groups, and can be separated by virtue of their hydrophobicity.

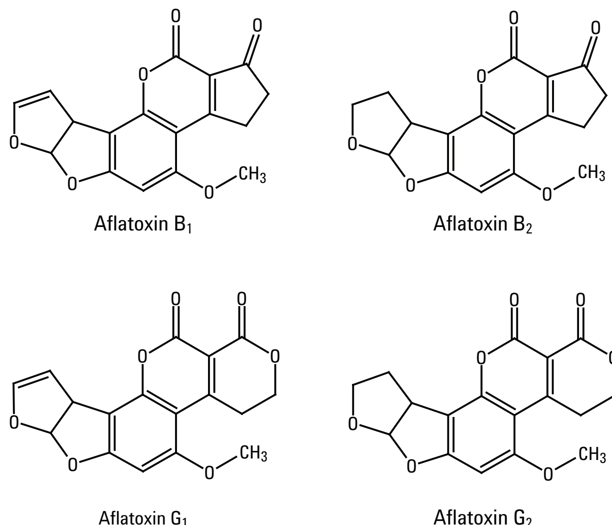

Figure 1. Chemical structures of target aflatoxins.

HPLC Methodology

While thin-layer chromatography was frequently used in the past, HPLC has been used in recent years because of its ease of operation and better quantitation. Most HPLC methods published to date have used reversed-phase HPLC on C18 bonded phases [1–4], where the aflatoxins are separated by their hydrophobicity. Most published separations have been performed on 5-µm columns of 25-cm in length. The use of smaller particle size packings in shorter columns with faster separation times are now in vogue. These columns show that the same separations can be achieved in less time than on the longer columns with similar resolution. In the present study, we desired to show that using such a column can provide improved results compared to the older methods. See Figure 2.

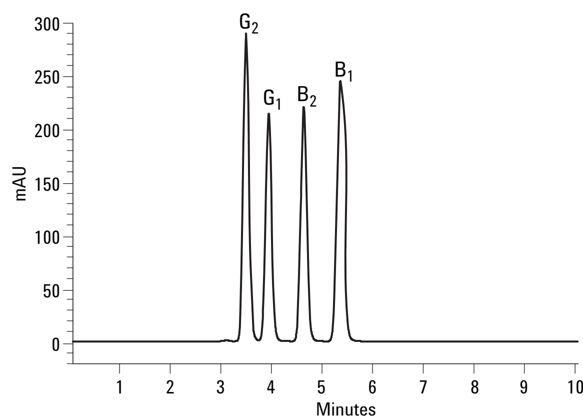

Figure 2. Reversed-phase separation of target aflatoxins using ZORBAX XDB-C18 Rapid Resolution column.

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Experimental Conditions

Chemicals:	The aflatoxins were purchased from Sigma Aldrich (Madrid, Spain).
HPLC Conditions	
Column:	ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 3.5 µm
Mobile phase:	Water/MeOH/ACN; 50/40/10 (V/V/V)
Flow rate:	0.8 mL/min
Temperature:	Ambient
Detector:	UV 365 nm
Injection volume:	10 µL (0.044 mg/mL)

Results and Discussion

All four aflatoxins were separated using an isocratic ternary mixture of water, methanol, and acetonitrile. A baseline separation was achieved in less than 5.5 min. Although UV detection was shown here, in some cases, lower levels of detection may be obtained for B_2 and G_2 using fluorescence (λ_{ex} = 365 nm, λ_{em} = 455 nm) detection. Mass spectroscopic detection has also been used [1].

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