SHIMADZU

Application News

High Performance Liquid Chromatography No.L422

High Speed, High Resolution Analysis (Part 39) Analysis of Aflatoxins in Food by Nexera

Aflatoxins are mycotoxins that are extremely carcinogenic and acutely toxic, and because they are subject to food contamination monitoring, their measurement is routinely conducted by HPLC and other analytical methods.
Ultra-high-speed analysis of aflatoxins in foods was previously introduced in Application News No. L351, in which aflatoxins B_1 and G_1 were analyzed using fluorescence detection following their conversion to hydroxyl-derivatives (B_{2a} and G_{2a}) using trifluoroacetic acid (TFA).
Here we introduce an example of ultra-high-speed analysis of aflatoxins in food using a combination of the Prominence RF-20Axs high-sensitivity fluorescence detector and the Nexera Ultra High Performance LC system, in which the aflatoxins B_1, B_2, G_1 and G_2 are analyzed using direct high-sensitivity fluorescence detection without conducting TFA derivatization.

■ Analysis of Aflatoxins Standard Solution

Fig. 1 shows a chromatogram of a standard mixture of the 4 aflatoxins, and Table 1 shows the analytical conditions. For the analytical column, the Shim-pack XR-ODS Ⅱ (100 mm L. × 3.0 mm I.D., 2.2 µm) was used. The concentrations of B_1 and G_1 were each 20 ng/L (20 ppt), and those of B_2 and G_2 were each 5 ng/L (5 ppt). The mixture was prepared in a solution of water / acetonitrile = 9/1 (v/v).
Use of the RF-20Axs allowed high sensitivity detection of B_1 and G_1 without undergoing derivatization with TFA.

Table 1 Analytical Conditions

Column	: Shim-pack XR-ODSⅡ (100 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: Water / Methanol / Acetonitrile = 6 / 3 / 1 (v/v/v)
Flow Rate	: 1.0 mL/min
Column Temp.	: 50 °C
Injection Volume	: 8 µL
Detection	: RF-20Axs Ex. at 365 nm, Em. at 450 nm
RF Cell	: Conventional cell
Cell Temp.	: 25 °C

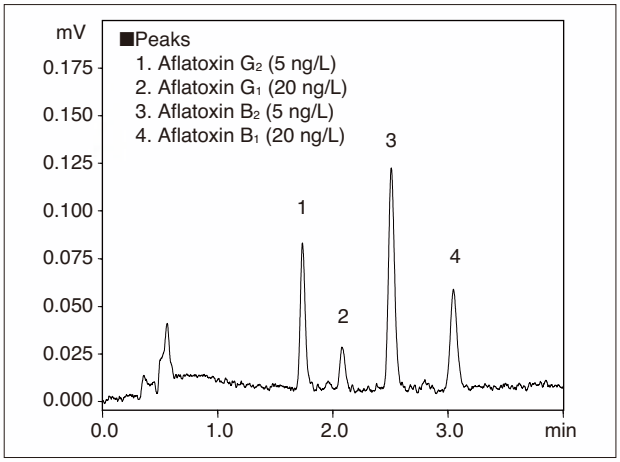

Fig. 1 Chromatogram of a Standard Mixture of Aflatoxins (8 µL injected)

Although an injection volume of 8 µL was used in the analysis shown in Fig. 1, we investigated the peak shapes and separation performance using larger sample injection volumes for analyses at even lower trace levels.
Fig. 2 shows the chromatograms of the same standard mixture of 4 aflatoxins used in the analysis of Fig. 1, but with injection volumes of 8 µL, 30 µL, and 50 µL, respectively.
Even with a 50 µL injection volume, good separation was obtained without any degradation of peak shape.
The detection limits (with SN ratio = 3.3) using the 50 µL injection were 1 ng/L (1 ppt) for aflatoxin B_1, and 2 ng/L (2 ppt) for aflatoxin G_1.

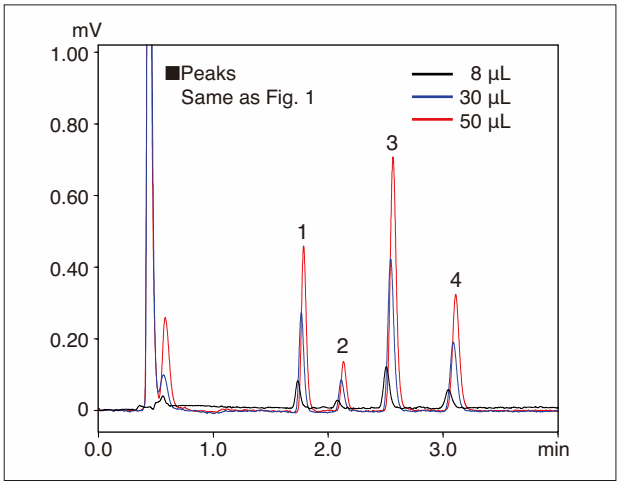

Fig. 2 Chromatograms of a Standard Mixture of Aflatoxins -Comparison of Injection Volume

■ Effect of Cell Temperature Control

It is generally known that fluorescence intensity is easily affected by the surrounding temperature, specifically that it is diminished or “quenched” at higher temperatures. Fig. 3 shows the relationship between the RF-20Axs cell temperature and the peak height ratio (based on a peak height of 1 at 20 °C).
From these results, it is clear that aflatoxins B_1 and B_2 are particularly affected by room temperature fluctuation. Because the RF-20Axs is equipped with a cell temperature control feature as standard, high accuracy analysis is possible without the adverse effect of room temperature fluctuation, even in the high-sensitivity analysis of aflatoxins.

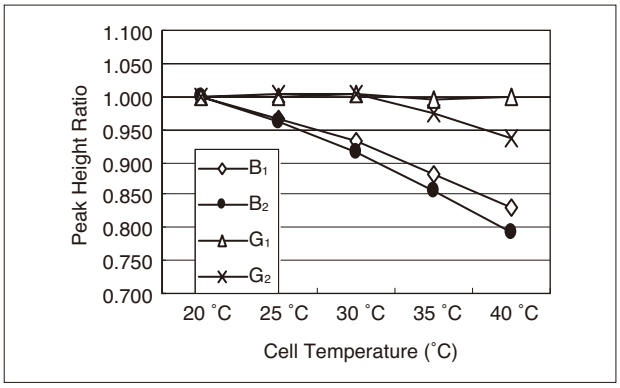

Fig. 3 Relationship between Cell Temperature and Peak Height Ratio

■ Analysis of Food Sample

We conducted sample preparation of commercially-available wheat flour according to the procedure shown in Fig. 4. In addition, the same preparation was also conducted for the same flour, but spiked with a standard mixture of 4 aflatoxins (B_1 and G_1 at 0.8 µg/kg, and B_2 and G_2 at 0.2 µg/kg). These samples were analyzed using the same analytical conditions as shown in Table 1 on the previous page. Fig. 5 shows the respective chromatograms.

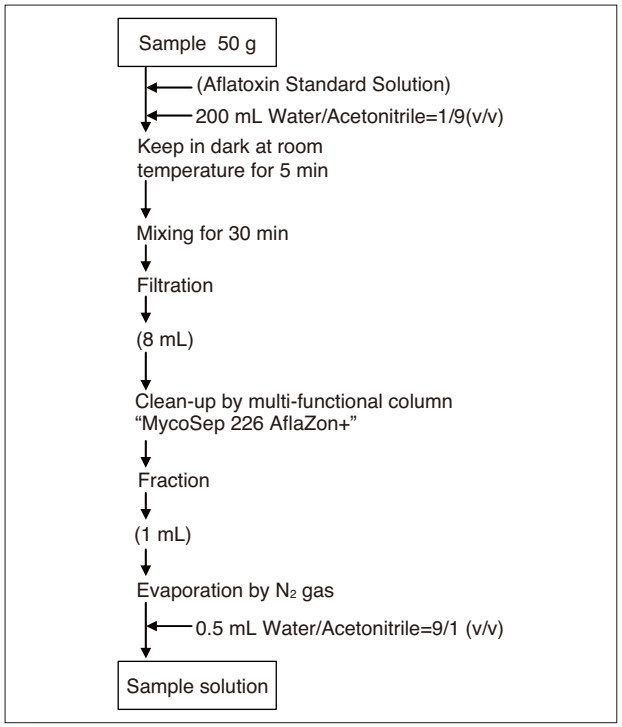

Fig. 4 Sample Preparation

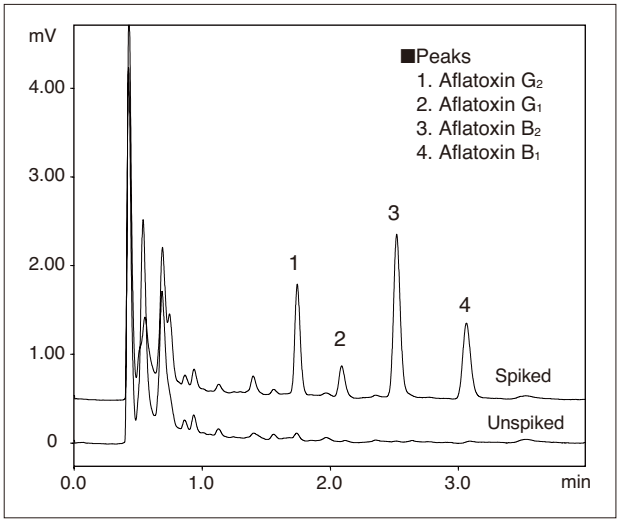

Fig. 5 Chromatograms of Wheat Flour Samples (8 µL injected) (Upper: Spiked, Lower: Unspiked)

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