SHIMADZU

**Nexera** Ultra High Performance Liquid Chromatograph

Nexera Application Data Sheet No.17

Ultrafast, Ultrahigh-Sensitivity Analysis of Aflatoxins

Aflatoxins are mycotoxins with a carcinogenic property and acute toxicity. They are measured by fluorescence detection HPLC and other instruments in order to prevent contamination of foods. Among four components (B_1, B_2, G_1, G_2) quantitated as the total aflatoxin, sensitivity for B_1 and G_1 is generally enhanced by a derivatization process using trifluoroacetic acid. Using the RF-20Axs High-Sensitivity Fluorescence Detector enables these components to be directly detected at a high sensitivity without any derivatization process. This document introduces an example of ultrafast, high-sensitivity analysis performed on aflatoxins using Nexera and the RF-20Axs.

Analysis of four aflatoxins

Four aflatoxin standard mixtures (B_1/G_1: 20 ng/L each, B_2/G_2: 5 ng/L each, water/acetonitrile = 9/1 solution) were analyzed using the Shim-pack XR-ODS II column (particle size 2.2 µm). Although the injection volume was 8 µL in this analysis, good separation was also achieved with an injection volume of 50 µL. When the injection volume was 50 µL, the detection limit (SN ratio = 3.3) was 1 ng/L (1 ppt) for B_1 and 2 ng/L (2 ppt) for G_1.
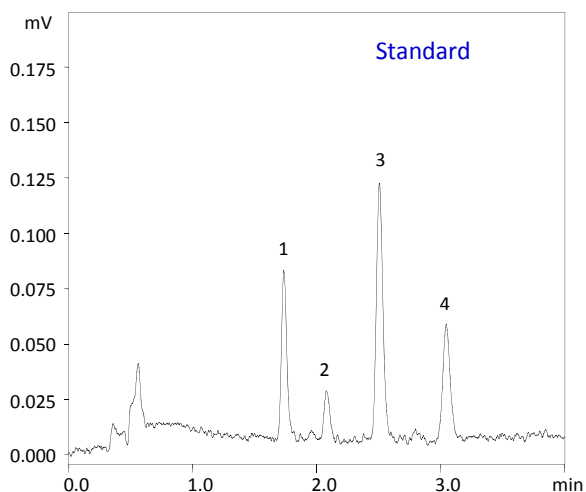
Column	: Shim-pack XR-ODS Ⅱ
	(100 mmL. x 3.0 mmI.D., 2.2 µm)
Mobile phase	: Water/Methanol/Acetonitrile=6/3/1(v/v/v)
Flow rate	: 1.0 mL/min
Column temp.	: 50 °C
Injection volume	: 8 µL
Detection	: Fluorescence (RF-20Axs)
	Ex. 365 nm, Em. 450 nm
Flow cell	: Conventional cell
Cell temp.	: 25 °C

Peaks:
1. Aflatoxin G_2 (5 ng/L, 5 ppt)
2. Aflatoxin G_1 (20 ng/L, 20 ppt)
3. Aflatoxin B_2 (5 ng/L, 5 ppt)
4. Aflatoxin B_1 (20 ng/L, 20 ppt)

Analysis of food samples

Four standard aflatoxins were added to commercially available wheat flour in amounts of 0.8 µg/kg for B_1/G_1 and 0.2 µg/kg for B_2/G_2. The samples were pretreated as shown in the procedure below and analyzed.

Sample 50 g
← (Aflatoxin standard solution)
← 200 mL Water/Acetonitrile=1/9 (v/v)
Leaving in dark
at room temperature, 5 min

Mixing 30 min

Filtration

(8 mL)

Clean-up by multi-functional column
"MycoSep 226 AflaZon+"

Fraction

(1 mL)

Evaporation by N_2 gas
← 0.5 mL Water/Acetonitrile=9/1 (v/v)

Sample solution

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