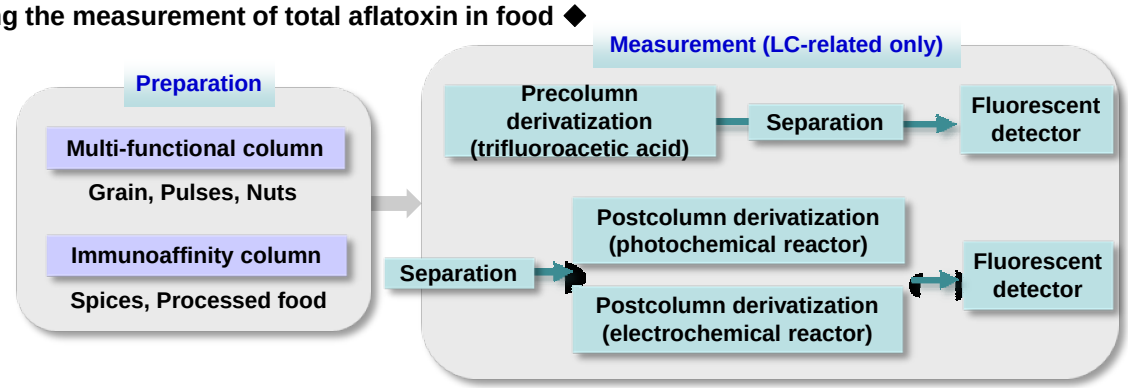
■ Measurement of Total Aflatoxin in Food

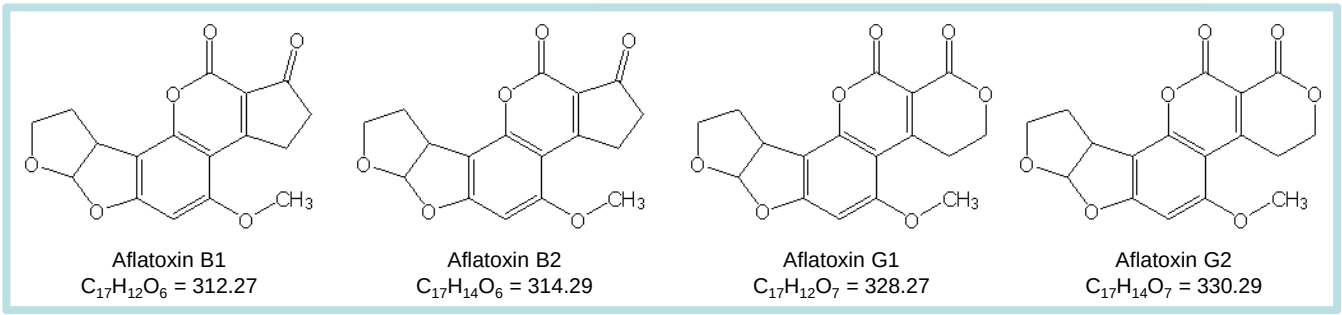
AS/LC-023

Among the mycotoxins (fungal toxins) that contaminate food, aflatoxin (AF) is a hepatotoxin and is considered to be the strongest naturally occurring carcinogen. Aflatoxins B1, B2, G1, and G2 exist in nature, and B1 is the strongest carcinogen. In Japan, the index for aflatoxin contained in food was changed from aflatoxin B1 to total aflatoxin (B1, B2, G1, and G2), and it is supposed to be under 10 µg/kg.*1 The regulation values have been set according to the individual circumstances in each country.
Samples are purified either using multi-functional column (MFC) or immunoaffinity column (IAC), depending on the type of each sample. Due to the low sensitivity of aflatoxins B1 and G1, derivatization is required for both standard and targeted samples. Samples are normally derivatized using trifluoroacetic acid with separation and detection by LC-FL, however, derivatization using photochemical reactors after column separation or electrochemical derivatization are also available for testing.*2
We hereby introduce the method of precolumn derivatization with trifluoroacetic acid and sample measurement by LC-FL.

*1 Notice No. 0331 Article 5 of the Department of Food Safety, Ministry of Health, Labour and Welfare of Japan, Mar. 31, 2011.
*2 Notice No. 0816 Article 1 of the Department of Food Safety, Ministry of Health, Labour and Welfare of Japan, Aug. 16, 2011.

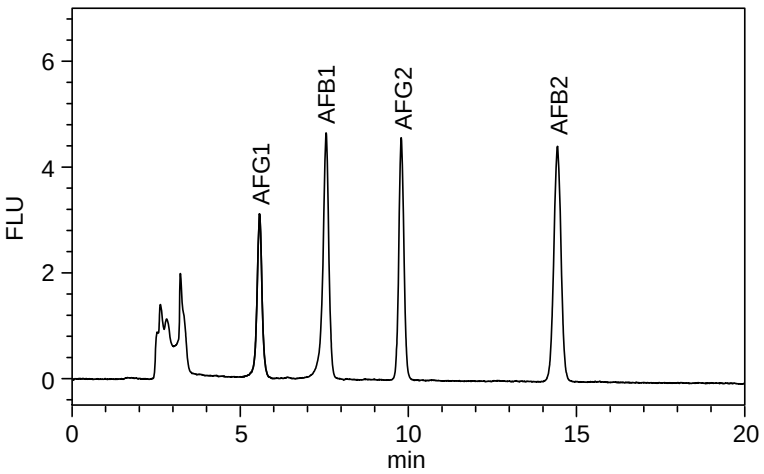
◆Introducing the measurement of total aflatoxin in food ◆





[Structural formula of aflatoxins]

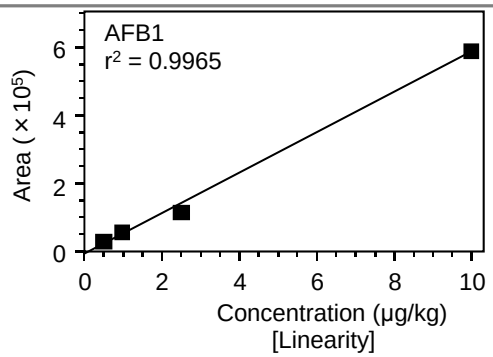
■ Aflatoxin (B1, B2, G1, and G2) Measurement of Standard Samples



[Measurement of standard samples (0.5 ng/mL (1.0 µg/kg) each]

<Analytical Conditions>

Column	:	LaChrom C18 (3 µm) 4.6 mm I.D. × 150 mm
Eluent	:	Acetonitrile/Methanol/H_2O= 1/3/6(v/v)
Flow Rate	:	0.7 mL/min
Column Temperature	:	40°C
Detection Wavelength	:	FL Ex 365 nm, Em 450 nm
Injection Vol.	:	20 µL



[Linearity]

Chromatogram of the minimum quantifiable value (1.0 µg/kg) of the method is shown. This data indicates the applicability of the method for testing. The contribution ratio is 0.997 within the linearity range of 0.5 - 10 µg/kg.

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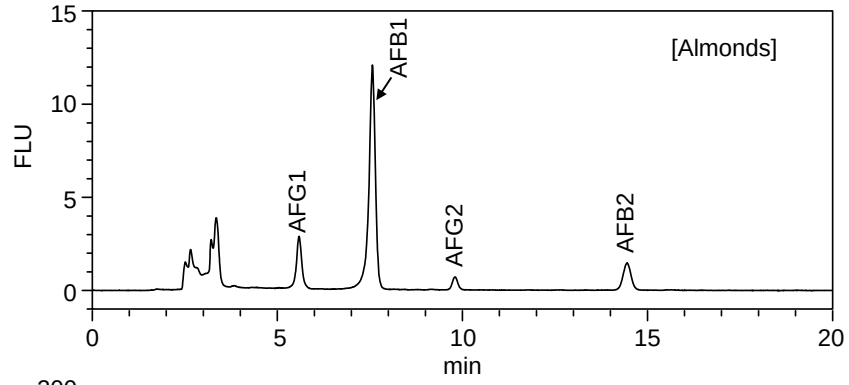
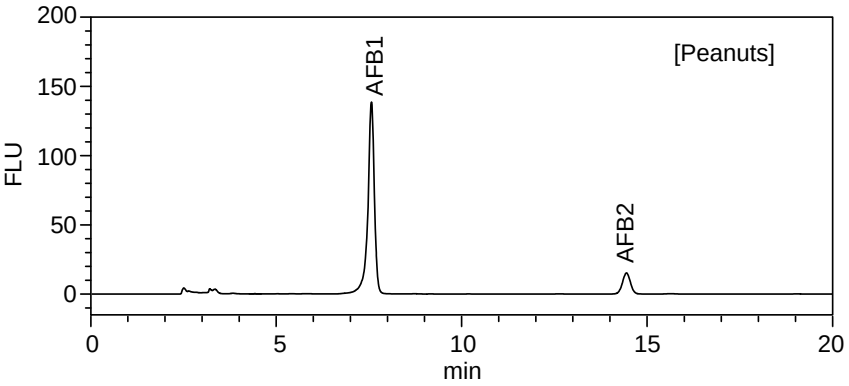
Measurement results of samples purified from a multi-functional column (MFC) and an immunoaffinity column (IAC) are shown. Derivatization of each sample was conducted by the precolumn method with trifluoroacetic acid.

■ Results of MFC analysis measurements of almonds and peanuts

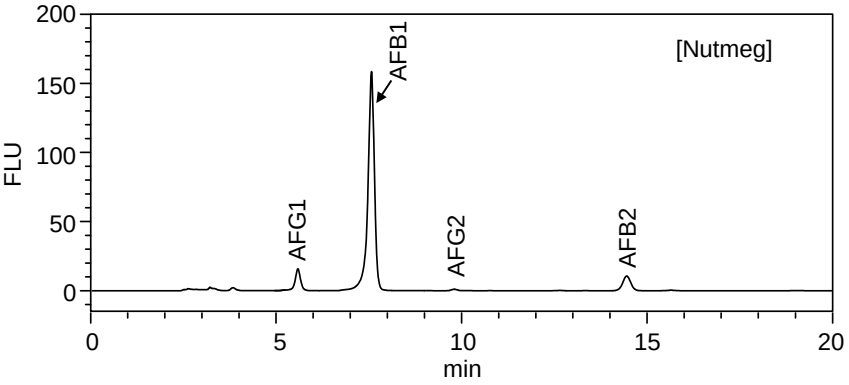


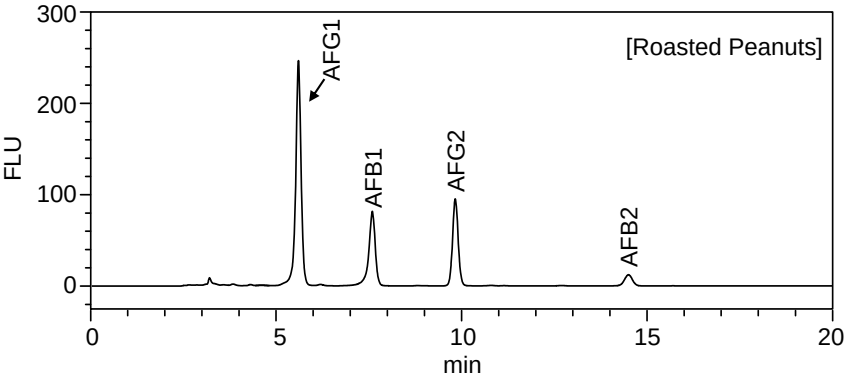


<Sample Preparation>

Sample 50 g
| ← H_2O + Acetonitrile (1 + 9) 200 mL
Homogenization followed by centrifugation
|
Filtrate 5 mL
|
Purification with solid phase extraction cartridges (MFC)
|
Elution Fractionation 2 mL
|
Drying under a nitrogen stream
| ← Trifluoroacetic acid 0.1mL
| Leave sample to stand for 15 min at room temperature in the dark
| ← Acetonitrile + H_2O (1 + 9) 0.9 mL
Analysis Sample (20 µL)

■ Results of IAC analysis measurements of nutmeg and roasted peanuts





<Sample Preparation>

Sample 50 g
| ← NaCl 5 g
| ← H_2O + Methanol (1 + 4) 200 mL
Homogenization followed by centrifugation
|
Filtrate 10 mL + H_2O 40 mL
|
Sample Solution 10 mL
|
Purification with AC
|
Elution Fractionation
|
Drying under a nitrogen stream
| ← Trifluoroacetic acid 0.1 mL
| Leave sample to stand for 15 min at room temperature in the dark
| ←
Acetonitrile + H_2O (1 + 9) 0.9 mL
Analysis Sample (20 µL)

* Samples for analysis were provided by Mycotoxin Research Association.

Major components of the instrument: Chromaster 5110 Pump, 5210 Autosampler (with thermostat), 5310 Column Oven, 5440 Fluorescence Detector

Note: These data are an example of measurement; the individual values cannot be guaranteed.