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Application News

No.L512

High Performance Liquid Chromatography

Analysis of Mycotoxins in Grain Using Mycotoxin Screening System

Mycotoxins are chemical products produced by organisms in the fungus kingdom and are toxic to humans, animals, and crops. As an example, aflatoxins are a type of mycotoxin that are some of the most carcinogenic naturally occurring substances in the world. They are classified as Group 1 carcinogens (carcinogenic to humans) by the WHO International Agency for Research on Cancer (IARC), and subject to strict regulations in many countries and regions of the world.
This Application News describes the screening analysis for mycotoxins in grain products (soft wheat flour and rice flour) using the i-Series Solution Package mycotoxin screening system.

i-Series Solution Package Mycotoxin Screening System

The screening system comprises a compact and easy to use integrated i-Series HPLC system together with analysis methods including sample pretreatment methods. The system comes ready to use and capable of data acquisition and analysis, including columns and method files designed for mycotoxin analysis, an instruction manual with analysis methods, and report templates. For screening applications, the system can determine whether mycotoxin levels in food are in excess of reference levels.

Nexera-i 3D, RF-20Axs

Fig. 1 Mycotoxin Screening System

Currently, HPLC and LC/MS are the most common techniques used to identify aflatoxins in food. With HPLC, fluorescent derivatization is often performed to improve sensitivity, though disadvantages of derivatization procedures are the time required and their complexity. Meanwhile, though LC/MS is more selective in terms of sensitivity, major financial investment into system is required.
The i-Series Solution Package comes with a built-in PDA detector, and can be further enhanced with an RF-20Axs fluorescence detector that offers world-class sensitivity. The package can also detect aflatoxins directly without derivatization.

Analysis of a Standard Solution

Mycotoxin targets of the screening system are shown in Table 1, chemical structures of some of these targets are shown in Fig. 2, and analytical conditions are shown in Table 2. The package includes analysis method files that eliminate the need to configure analytical conditions. An RF-20Axs was used to perform analysis with on-time excitation wavelength/emission wavelength switching.

Table 1 Screening Target Compounds

	Mycotoxin	Abbreviation	Matrix
1	Aflatoxin M_1	AFM_1	Milk
2	Aflatoxin G_2	AFG_2	Grain
3	Aflatoxin G_1	AFG_1	
4	Aflatoxin B_2	AFB_2	
5	Aflatoxin B_1	AFB_1	
6	Zearalenone	ZON	
7	Ochratoxin A	OTA	
8	Nivalenol	NIV	
9	Deoxynivalenol	DON	
10	Patulin	PAT	Apple

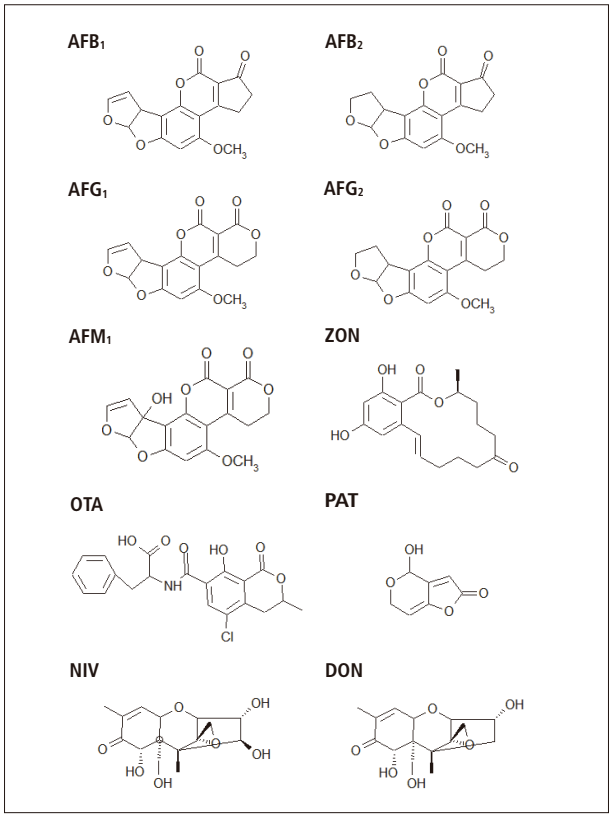

Fig. 2 Target Mycotoxin Structures

Table 2 Analytical Conditions

System	:	Nexera-i 3D, RF-20Axs
Column	:	Shim-pack GIST C18 (75 mm L. × 3.0 mm I.D., 2 µm)
Mobile Phase	:	A) 20 mmol/L (Sodium) phosphate buffer (pH 2.5) B) Acetonitrile C) Methanol (Gradient elution)
Flowrate	:	1.0 mL/min
Column Temp.	:	55 °C
Injection Vol.	:	10 µL
Detection (RF-20Axs)	:	AFB1, AFB2, AFG1, AFG2, AFM1 : Ex 365 nm, Em 450 nm OTA, ZON : Ex 320 nm, Em 465 nm
Detection (Nexera-i 3D)	:	NIV, DON : 220 nm (ch 1) PAT : 276 nm (ch 2)

Although regulatory limits for mycotoxin levels in food can vary by country and region, the screening system is compatible with the strictest regulatory limits that are found in the EU (excluding regulatory limits in baby food). Chromatograms of a standard mixture with mycotoxin levels equivalent to EU reference levels[1] is shown in Fig. 3.

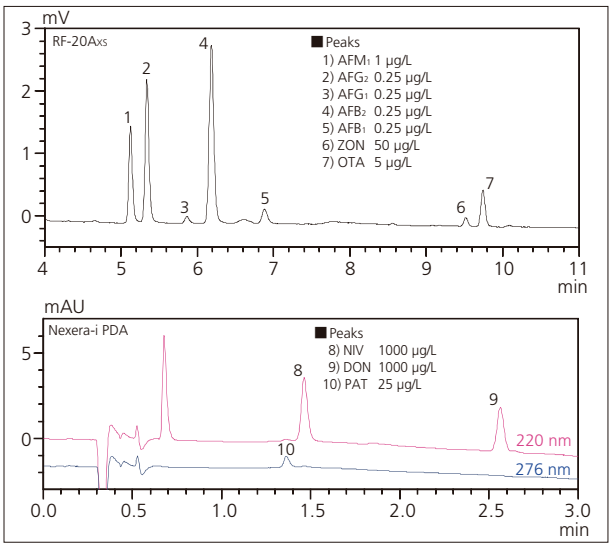

Fig. 3 Chromatograms of a Standard Mixture

Analysis of Mycotoxins in Grain

This section describes an analysis of milled grains after pretreatment. Fig. 4 shows an overview of the sample pretreatment method. Further details can be found in the mycotoxin screening system instruction manual. Chromatograms of pretreated samples of soft wheat flour and rice flour and of pretreated samples of soft wheat flour and rice flour spiked with a standard mixture of mycotoxins that are produced in grains (shown among the screening target compounds listed in Table 1) are shown in Fig. 5 and Fig. 6.

Comparing the area of each peak in the standard mixture that contains mycotoxins at EU reference levels and each peak in the flour samples allows identification of whether the mycotoxins present in flour samples are in excess and violation of reference levels. The system makes this determination without the need for complex analysis of results by the user, allowing for easy screening of target compounds.

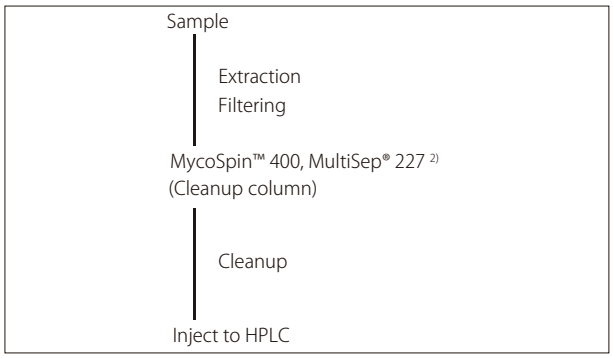

Fig. 4 Sample Pretreatment

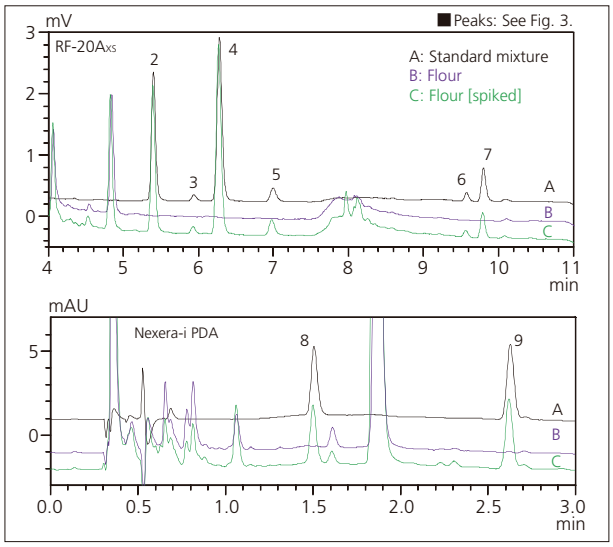

Fig. 5 Chromatograms of Soft Wheat Flour

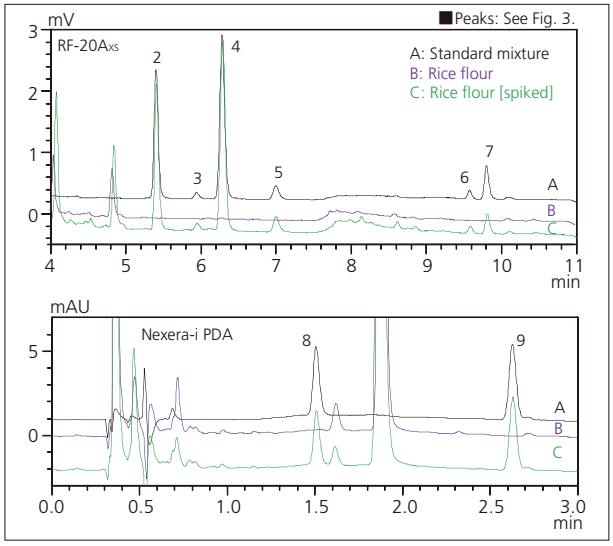

Fig. 6 Chromatograms of Rice Flour

Footnotes

1) Converted concentrations in the standard mixture were obtained according to a pretreatment method described in the i-Series Solution Package Mycotoxin Screening System instruction manual.
2) MycoSpin™ 400 and MultiSep® 227 are registered trademarks of Romer Labs.

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