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Application News

No.L484

High Performance Liquid Chromatography

Analysis of Nivalenol and Deoxynivalenol in Wheat Using Prominence-i

Nivalenol and deoxynivalenol (DON, vomitoxin) are types of mycotoxins produced by Fusarium fungi. In Japan, the provisional reference value for deoxynivalenol was set at 1.1 ppm in May, 2002 (Notification No. 0521001 issued by Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan).
Previously, in Application News No. L362, the analysis using an ultra high-performance LC system was introduced, but here, referring to the test method for deoxynivalenol specified in the Notification No. 0717001 (issued by Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, in July 2003), a washing process for the analytical column is included.
The detector component of the new Prominence-i integrated high-performance liquid chromatograph incorporates a temperature control function for both the flow cell component and the optical system. Here, good repeatability was obtained despite the susceptibility of UV detection in the short wavelength region due to environmental temperature fluctuations.

Analysis of Standard Mixture

Fig. 1 shows the results of analysis of a standard mixed solution of nivalenol and deoxynivalenol (each at 4.0 ppm) using a 10 µL injection. Table 1 shows the analytical conditions used. The test method specifies the use of isocratic analysis, but a column washing process after elution of the deoxynivalenol was added. As the Prominence-i is equipped with a low-pressure gradient unit as standard, a mobile phase with a high organic solvent ratio can easily be pumped through the system following elution of the target component.
Six repeat analyses of a 0.1 ppm standard solution were conducted, corresponding to about one-tenth the provisional reference value. The relative standard deviation (% RSD) of peak area and retention time obtained for the two substances are shown in Table 2, and the chromatogram is shown in Fig. 2.

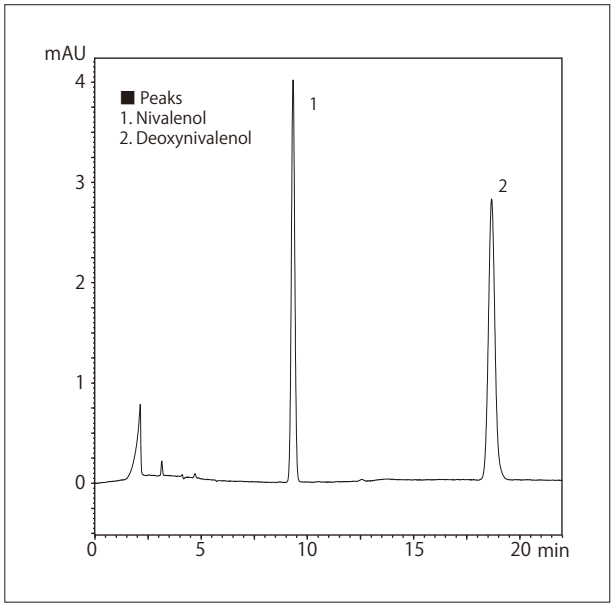

Fig. 1 Chromatogram of a Standard Mixture (4.0 ppm each)

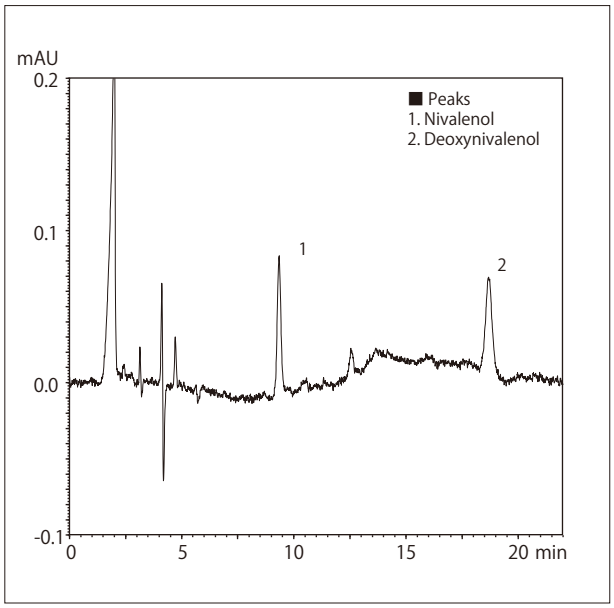

Fig. 2 Chromatogram of a Standard Mixture (0.1 ppm each)

Table 1 Analytical Conditions

Column	: Shim-pack GIS C18 (250 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase A	: Water / Acetonitrile / Methanol = 90/5/5 (v/v/v)
Mobile Phase B	: Acetonitrile / Methanol = 50/50 (v/v)
Time Program	: B Conc. 0 % (0 - 20 min) → 50 % (20.01 - 25 min) → 0 % (25.01 - 45 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 10 µL
Detection	: UV 220 nm (Cell temp. 45 ˚C)

Table 2 Repeatability (0.1 ppm, n=6)

	R.T.%RSD	Area %RSD
Nivalenol	0.09	0.68
Deoxynivalenol	0.06	0.76

Calibration Curve Linearity

Fig. 3 shows the calibration curves generated from analyses using the conditions of Table 1. Excellent linearity with a coefficient of determination greater than R^2=0.9999 was obtained for both substances over a concentration range of 0.1 to 4 ppm.

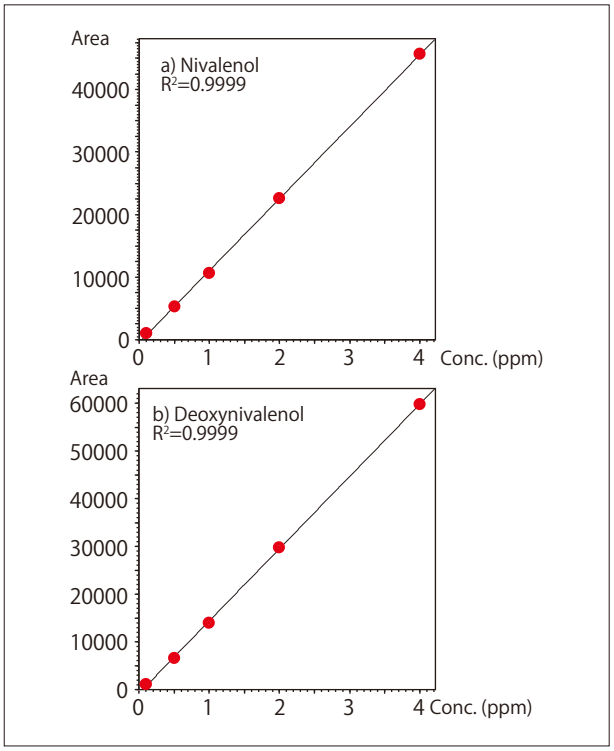

Fig. 3 Linearity of Calibration Curves for a) Nivalenol and b) Deoxynivalenol

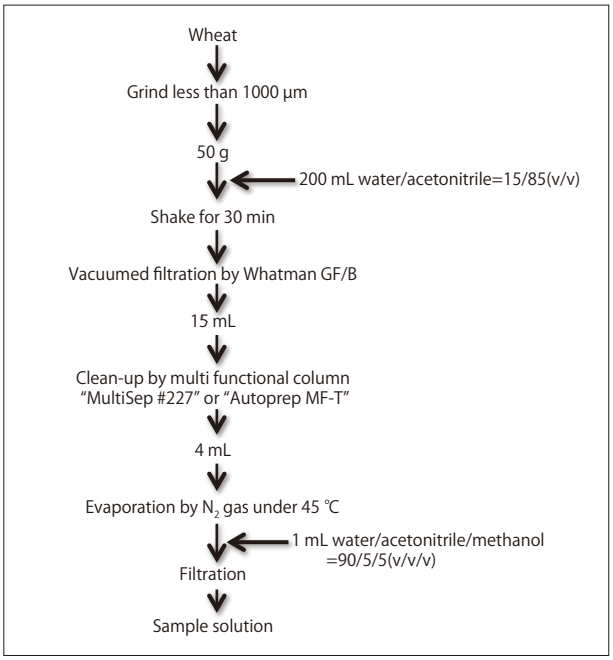

Fig. 4 Pretreatment

Analysis of Wheat

Fig. 4 shows the pretreatment procedure used for analysis of wheat. Purification of two varieties of wheat was conducted using two types of multi-function columns, the "MultiSep #227" (Romer Labs Inc.) and "Autoprep MF-T" (Showa Denko K.K.).

The results are shown in Fig. 5 and Fig. 6, respectively. Here, the pretreated sample solution was spiked with nivalenol and deoxynivalenol to achieve respective concentrations of 1.0 ppm.

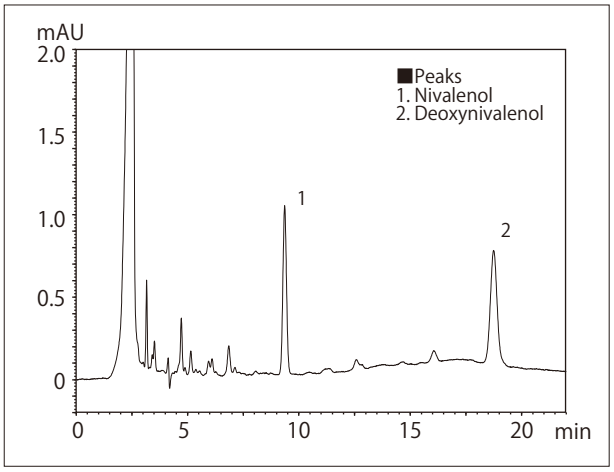

Fig. 5 Chromatogram of Wheat A (Spiked with 1.0 ppm each) (MultiSep #227)

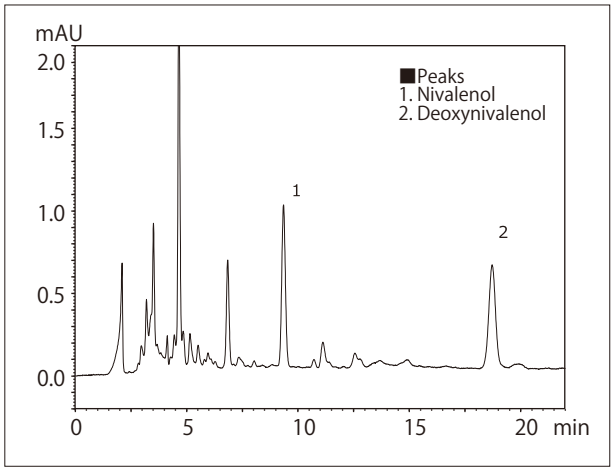

Fig. 6 Chromatogram of Wheat B (Spiked with 1.0 ppm each) (Autoprep MF-T)

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