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Application News

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Liquid Chromatography Mass Spectrometry

Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Nivalenol and deoxynivalenol are mycotoxins which are produced by the fusarium fungi. A provisional reference value of 1.1 ppm was established in Japan for deoxynivalenol (Notification No. 0521001 issued by the Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on May 21, 2002). The test methods specified for deoxynivalenol are HPLC for both qualitative and quantitative analysis, and LC/MS for verification testing (Notification No. 0717001 issued by the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on July 17, 2003).
This paper describes an LC-MS/MS method for high-sensitivity simultaneous analysis of the four compounds, nivalenol, deoxynivalenol and the deoxynivalenol metabolytes, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol.

Analysis of a Standard Mixture

Fig. 1 shows the chromatograms obtained using a 2 µL injection of the four-component standard mixture (each 10 ppb), and Table 1 shows repeatability of retention time and peak areas for the four substances, respectively, using six repeat measurements.
Nivalenols are detected using the heated electrospray ionization (hESI) method in negative mode. Although water and acetonitrile alone can be used as the LC eluent for LC/MS analysis, higher sensitivity was obtained for each compound by adding low-concentration ammonium acetate (in this case, 0.5 mmol/L) to eluent A. Fig. 1 shows the mass chromatograms for the highest sensitivity MRM transitions for each compound. The analytical conditions are shown in Table 2.
Next, six repeat analyses of a 10 ppb standard solution were conducted, corresponding to approximately 1/100 the concentration of the provisional reference value. The relative standard deviations (%RSD) for the measured retention times and peak areas are shown in Table 1. Good repeatability was obtained for both retention time and peak area.

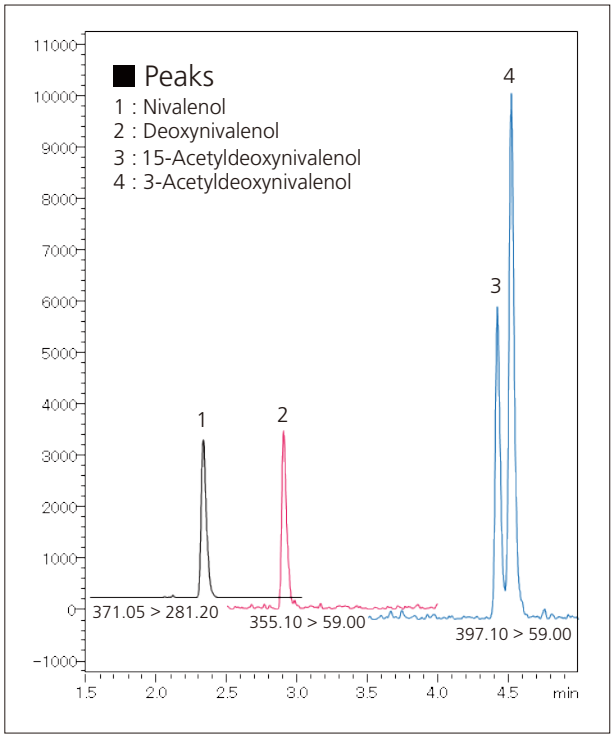

Fig. 1 MRM Chromatograms of a Standard Mixture (10 ppb each)

Table 1 Repeatability (10 ppb, n=6)

	R.T. %RSD	Area %RSD
Nivalenol	0.04	2.57
Deoxynivalenol	0.04	6.52
15-Acetyldeoxynivalenol	0.06	4.09
3-Acetyldeoxynivalenol	0.05	2.58

Linearity of Calibration Curves

Fig. 2 shows the calibration curves generated using the analytical conditions of Table 2. Excellent linearity with a coefficient of determination greater than $R^2 = 0.999$ was obtained for calibration curves using a concentration range from 1 to 250 ppb for each component.

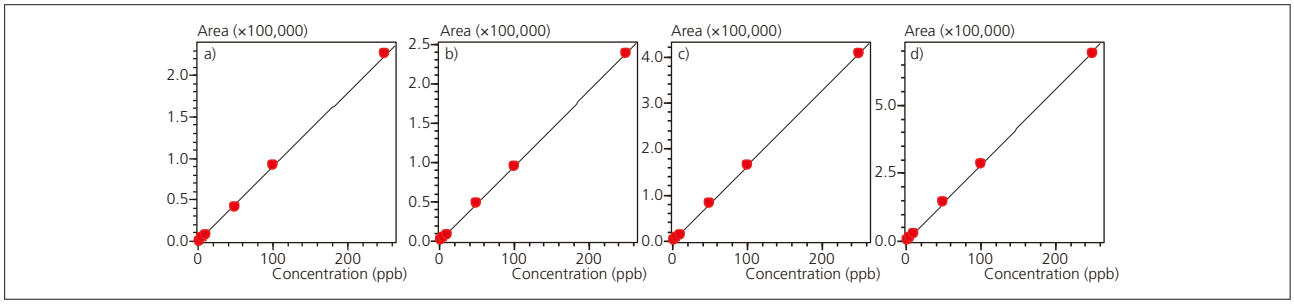

Fig. 2 Linearity of Calibration Curves: a) Nivalenol b) Deoxynivalenol c)15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

Analysis of Wheat

Fig. 3 describes the sample pretreatment procedure for wheat. The wheat extract solution was purified using either the MultiSep #227 multi-function column (Romer Labs) or the Autoprep MF-T column (Showa Denko K.K.). The chromatograms generated using the samples prepared using the MultiSep #227 (unspiked samples) and the standard-spiked samples, respectively, are shown in Fig. 4. The standard mixture was added to obtain a final concentration of 25 ppb for the four components (about 1/40 of the provisional reference value), respectively. No large contaminant peaks were detected in the chromatograms of the pretreated samples. Furthermore, although deoxynivalenol was detected, it was at a level below that of the provisional reference value. The spike-and-recovery rates for the four components were excellent, from 101 to 107 %, without any particular matrix effects. Even in samples pretreated using Autoprep MF-T, comparable spike-and-recovery test results were obtained.

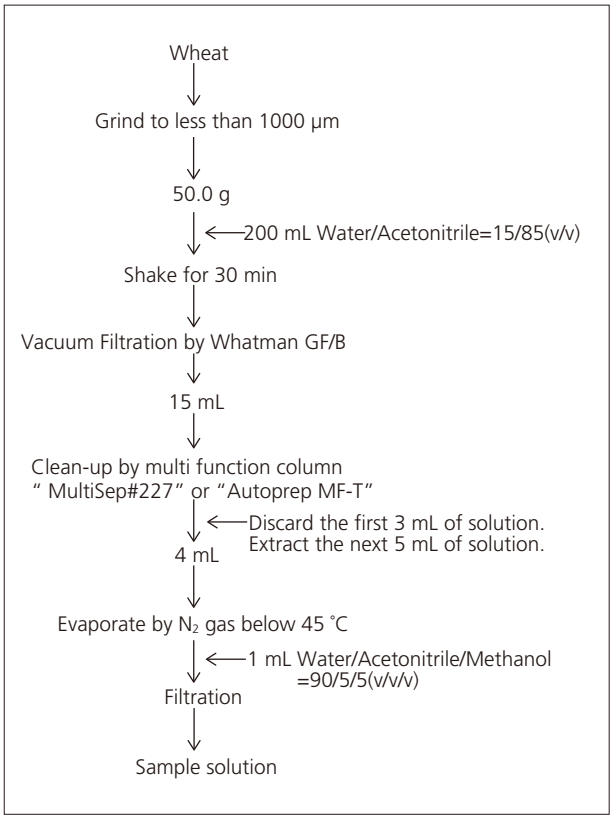

Fig. 3 Pretreatment

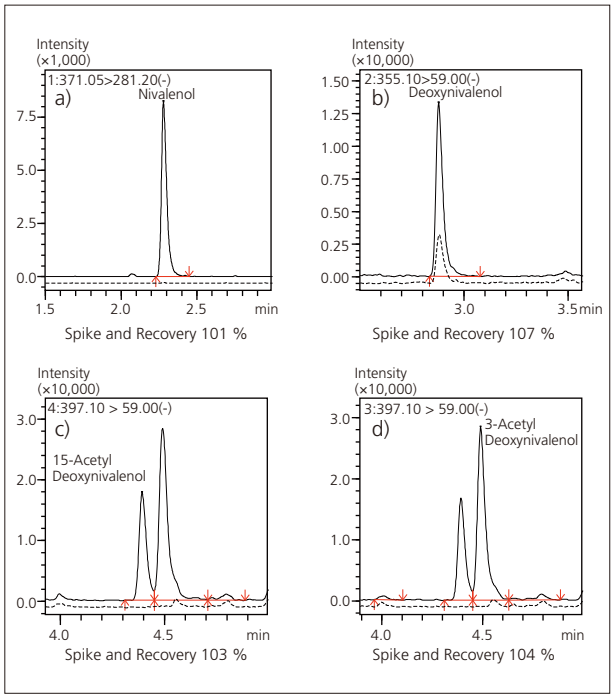

Fig. 4 Chromatograms of Wheat
(Dotted line: Unspiked Sample, Solid line: Spiked Sample, Spiked at 25 ppb each)
a) Nivalenol b) Deoxynivalenol
c) 15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

Table 2 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅲ (150 mm L. × 2.0 mm I.D., 2.2 µm)		
Mobile Phases	: A 0.5 mmol/L Ammonium Acetate - Water		
	: B Acetonitrile		
Time Program	: 5 %B (0 min) → 45 %B (5.0 min) → 95 %B (5.01-7.0 min) → 5 %B (7.01 min) → STOP (12 min)		
Flowrate	: 0.3 mL/min		
Column Temperature	: 40 ˚C		
Injection Volume	: 2 µL		
Probe Voltage	: -3.0 kV (ESI-negative mode)		
DL Temperature	: 100 ˚C		
Block Heater Temperature	: 200 ˚C		
Interface Temperature	: 200 ˚C		
Nebulizing Gas Flow	: 2 L/min		
Drying Gas Flow	: 10 L/min		
Heating Gas Flow	: 10 L/min		
MRM Transition	: Nivalenol	371.05 > 281.20	CE: 16.0 V
	: Deoxynivalenol	355.10 > 59.00	CE: 22.0 V
	: 15-Acetyldeoxynivalenol	397.10 > 59.00	CE: 22.0 V
	: 3-Acetyldeoxynivalenol	397.10 > 59.00	CE: 26.0 V

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