Amended, Sep 10, 2021
Implemented, Jan 1, 2022
MOHWT0015.01

# Method of Test for Mycotoxins in Foods -Test of Deoxynivalenol (2)

## 1. Scope

This method is applicable to the determination of deoxynivalenol in grains, cereal based foods for infant and young child and baby food.

## 2. Method

After extraction, deoxynivalenol is determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

**2.1.** Equipment

**2.1.1.** Liquid chromatograph/tandem mass spectrometer.

**2.1.1.1.** Ion source: electrospray ionization, ESI.

**2.1.1.2.** Column: Inertsil ODS-3, 3 μm, 2.1 mm i.d. × 15 cm, or an equivalent product.

**2.1.2.** Vortex mixer.

**2.1.3.** Shaker.

**2.1.4.** Centrifuge: centrifugal force ≥ 3000 ×g.

**2.1.5.** Nitrogen evaporator.

**2.1.6.** Ultrasonicator.

**2.1.7.** Vacuum freeze-drying device: temperature ≤ -40°C, and degree of vacuum ≤ 133 mBar.

**2.1.8.** Oven: with an automatic temperature adjustment, capable of controlling temperature at ± 2°C.

**2.1.9.** Desiccator.

**2.1.10.** Grinder.

**2.1.11.** Blender.

**2.2.** Chemicals

Acetonitrile, HPLC Grade;
Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
Deoxynivalenol, reference standard.

**2.3.** Apparatus

**2.3.1.** Centrifuge tube: 50 mL, PP.

**2.3.2.** Volumetric flask: 1 mL and 10 mL.

**2.3.3.** Membrane filter: 0.22 μm, Nylon.

**2.3.4.** Weighing bottle: with cap.

**2.4.** Reagents

**2.4.1.** 20% acetonitrile

Dilute 20 mL of acetonitrile with deionized water to 100 mL.

**2.4.2.** 85% acetonitrile

Dilute 850 mL of acetonitrile with deionized water to 1000 mL.

**2.4.3.** 10% acetonitrile

Dilute 10 mL of acetonitrile with deionized water to 100 mL.

**2.5.** Standard solution preparation

Transfer about 1 mg of deoxynivalenol reference standard accurately weighed to a 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solution. Store under freezing. When to use, mix appropriate volume of the standard stock solution, and dilute with 10% acetonitrile to 50-250 ng/mL as the standard solutions.

**2.6.** Sample solution preparation

Transfer about 5 g of the homogenized sample(note) accurately weighed into a centrifuge tube. Accurately add 20 mL of 85% acetonitrile, shake for 60 min, and centrifuge at 3000 ×g for 5 min. Collect 4 mL of supernatant, and evaporate to dryness by gently flushing with a stream of nitrogen at 40°C. Dissolve and dilute the residue with 10% acetonitrile to 1 mL by sonicating until it is dissolved. Filter with a membrane filter, and take the filtrate as the sample solution.

Note: Cereal based foods for infant and young child and baby food should be freeze-dried to make the moisture content less than 10% before sample solution preparation.

**2.7.** Matrix-matched calibration curve

Take a blank sample, and follow the extraction and evaporation to dryness procedure described in section 2.6. Separately add 1 mL of the standard solution, sonicate for 10 min, and filter with a membrane filter as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of deoxynivalenol by the peak area of deoxynivalenol vs. the added concentrations.

LC/MS/MS operating conditions(note):

Column: Inertsil ODS-3, 3 μm, 2.1 mm i.d. × 15 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.0 → 10.0 | 95 → 40 | 5 → 60 |

| | | |
|---|---|---|
| 10.0 → 10.1 | 40 → 0 | 60 → 100 |
| 10.1 → 12.0 | 0 → 0 | 100 → 100 |
| 12.0 → 12.1 | 0 → 95 | 100 → 5 |
| 12.1 → 15.0 | 95 → 95 | 5 → 5 |

Flow rate: 0.4 mL/min.

Injection volume: 10 μL.

Capillary voltage: -4.5 kV.

Ion source: $ESI^-$.

Turbo heater temperature: 500°C.

Nebulizer gas (GS1): 50 psi.

Heated gas (GS2): 50 psi.

Curtain gas: 20 psi.

Collision gas: High.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, declustering potential and collision energy are shown as follows:

| Analyte | Ion pair<br>Precursor ion (*m/z*) > product ion (*m/z*) | Declustering potential (V) | Collision energy (eV) |
|---|---|---|---|
| Deoxynivalenol | 295 > 265* | -130 | -14 |
| | 295 > 138 | -120 | -14 |

*The quantitative ion.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

**2.8.** Moisture content determination

Transfer about 2 g of the pre-freeze-dried homogenized cereal based foods for infant and young child and baby food sample into a weighing bottle predried to constant weight ($m_0$), and weigh accurately ($m_1$). Place the weighing bottle in an oven, and dry at 105°C for 2 hr. Cap the weighing bottle, place in a desiccator to cool to room temperature (about 30 min), and weigh the weighing bottle. Replace the weighing bottle in the oven, dry for 1 hr, and weigh following the above procedure until constant weight ($m_2$). Calculate the moisture content of the sample by the following formula:

$$\text{The moisture content of the sample (\%)} = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where,

$m_0$: the weight of the weighing bottle with cap (g)

$m_1$: the weight of the weighing bottle with cap and the sample (g)

$m_2$: the weight of the weighing bottle with cap and the sample after drying to constant weight (g)

**2.9.** Identification and quantification

Accurately inject 10 μL of the sample solution and the matrix-matched standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.7. Identify deoxynivalenol based on the retention time and the relative ion intensities(note). Calculated the amount of deoxynivalenol in the sample by the following formula:

**2.9.1.** Grains

$$\text{The amount of deoxynivalenol in the sample (μg/kg)} = \frac{C \times V \times 5}{M}$$

Where,

C: the concentration of deoxynivalenol in the sample solution calculated by the matrix-matched calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

**2.9.2.** Cereal based foods for infant and young child and baby food

$$\text{The amount of deoxynivalenol in the sample (μg/kg)} = \frac{C \times V \times 5}{M \times (1 - W/100)}$$

Where,

C: the concentration of deoxynivalenol in the sample solution calculated by the matrix-matched calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

W: the moisture content of the sample (%)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities are as follows:

| Relative ion intensity (%) | Tolerance (%) |
|---|---|

| | |
|---|---|
| > 50 | ± 20 |
| > 20~50 | ± 25 |
| > 10~20 | ± 30 |
| ≤ 10 | ± 50 |

**Remark**

1. The limit of quantification (LOQ) for deoxynivalenol is 50 μg/kg.
2. Further validation should be performed when interfering compounds appear in samples.

**References**

1. García-Moraleja, A., Font, G., Mañes, J. and Ferrer, E. 2015. Development of a new method for the simultaneous determination of 21 mycotoxins in coffee beverages by liquid chromatography tandem mass spectrometry. Food Res. Int. 72: 247-255.
2. Wu, S. H., Chiu, J. Y., Yu, M. C., Lwo, C. H., Chang, T. P., Chen, J. H. and Shih, W. C. 2019. Development and validation of analytical methods for the determination of mycotoxins and PAHs in foods. Commissioned Research Report of Taiwan Food and Drug Administration.