NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of deoxynivalenol and its acetylated derivatives in food

食品安全国家标准 食品中脱氧雪腐镰刀菌烯醇及其乙酰化衍生物的测定

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1 Scope

The document gives methods for determining deoxynivalenol and its acetylated derivatives in food.

The first method, isotope dilution liquid chromatography-tandem mass spectrometry, applies to the determination of deoxynivalenol, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in cereals and cereal products, alcoholic drinks, soy sauce, vinegar, and pastes and paste products.

The second method, immunoaffinity chromatographic clean-up with high performance liquid chromatography, applies to the determination of deoxynivalenol in the same food groups.

The third method is thin-layer chromatographic determination and the fourth is enzyme-linked immunosorbent screening; both apply to the determination of deoxynivalenol in cereals and cereal products.

5 Analytical procedure, first method

5.1 Sample preparation. For cereals and cereal products at least 1 kg is taken, ground in a high speed mill and sieved so that the particle size passes a test sieve of 0.5 mm to 1 mm aperture, then mixed and reduced to 100 g and kept sealed. For alcoholic drinks at least 1 L of bulk product, or at least three packages of the same batch, is homogenised in one vessel and reduced to 100 g or mL; carbonated samples are refrigerated at 4 °C for 30 min and filtered or degassed by ultrasound before use. Soy sauce, vinegar, pastes and paste products are treated in the same way starting from at least 1 L.

5.2 Extraction. For cereals and cereal products 2 g are weighed to 0.01 g into a 50 mL centrifuge tube, 400 µL of the mixed isotope internal standard working solution is added, the tube is shaken and left 30 min, 20.0 mL of acetonitrile-water (84 + 16) is added and the tube is sonicated or shaken 20 min, then centrifuged 5 min at 10 000 r/min; the supernatant is kept as supernatant A. For alcoholic drinks 5 g are weighed, 200 µL of internal standard is added, the tube is left 30 min, made to 10 mL with acetonitrile and treated in the same way to give supernatant B. Soy sauce, vinegar, pastes and paste products follow the cereal procedure with 2 g and 400 µL and give supernatant C.

5.3 Clean-up. One of three routes is chosen. On the general purpose solid phase extraction

cartridge, 5 mL of supernatant is mixed with 10 mL of acetonitrile-saturated hexane, vortexed 2 min and centrifuged 2 min at 5 000 r/min, the hexane layer is discarded, the residue is blown to dryness with nitrogen at 40 °C to 50 °C and taken up in 4 mL of water; the cartridge is conditioned with 3 mL of methanol and 3 mL of water, the sample is loaded at one to two drops per second, the cartridge is washed with 3 mL of water and 1 mL of 5 % methanol in water and dried, and the analytes are eluted with 4 mL of methanol, blown dry, redissolved in 1.0 mL of initial mobile phase and filtered through a 0.22 μm membrane. On the dedicated cartridge, 8 mL of supernatant is placed in the glass tube, the packing tube is pushed in slowly until purified liquid appears, and 5 mL of it is blown dry and taken up as before. On the immunoaffinity column, 5 mL of supernatant is blown dry, taken up in 2 mL of water, passed through the column at one drop per second with an air pressure pump, washed with 5 mL of phosphate buffered saline and 5 mL of water, eluted with 2 mL of methanol at one drop per second, blown almost dry at 50 °C, taken up in 1.0 mL of initial mobile phase by vortexing 30 s and filtered.

5.4 Reference chromatographic and mass spectrometric conditions are given for a positive electrospray source and for a negative electrospray source. Both use a C18 column 100 mm long with an internal diameter of 2.1 mm and 1.7 μm packing, a flow rate of 0.35 mL/min, a column temperature of 40 °C, an injection volume of 10 μL, a desolvation gas flow of 900 L/h and the same gradient, 2 % B from 0 min to 0.8 min, 24 % B from 3.0 min to 4.0 min, 100 % B from 6.0 min to 6.9 min and 2 % B from 6.9 min to 7.0 min. In positive mode the mobile phases are 0.1 % formic acid and 0.1 % formic acid in acetonitrile, the capillary voltage is 3.5 kV, the cone voltage 30 V and the desolvation temperature 350 °C. In negative mode they are 0.01 % ammonia and acetonitrile, the capillary voltage is 2.5 kV, the cone voltage 45 V and the desolvation temperature 500 °C. Tables 1 and 2 list, for each compound, the precursor ion, the cone voltage, the quantifier ion and its collision voltage and the qualifier ion and its collision voltage; a note states that because 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol are isomers, the carbon-13 labelled 3-acetyl compound may be used as the internal standard for the 15-acetyl compound.

5.5 Identification. The retention time of the target peak in the sample varies from that of the corresponding standard peak by not more than +/- 2.5 %. The qualifying ions appear, at least one precursor and two product ions, and within one analytical batch the relative abundance ratio of the two product ions differs from that of a standard solution of comparable concentration by not more than the deviation given in Table 3, which allows +/- 20 % where the relative abundance is above 50 %, +/- 25 % from 20 % to 50 %, +/- 30 % from 10 % to 20 % and +/- 50 % at or below 10 %.

5.6 to 5.8 The calibration curve is drawn from the ratio of the peak area of each compound to that of its internal standard against concentration, and the linear correlation coefficient is greater than 0.99. The sample response falls inside the linear range, otherwise the test portion is reduced and the determination repeated. A blank test is run without the sample.

6 The content is calculated with formula (1). The formula as printed in the source is broken and is not reproduced here. Its symbols are the content of deoxynivalenol, 3-acetyl-deoxynivalenol or 15-acetyl-deoxynivalenol in the sample in micrograms per kilogram; the mass concentration read from the calibration curve by the internal standard method, in nanograms per millilitre; the volume of the sample extract, the final volume of the sample, and the aliquot volume taken for clean-up, all in millilitres; the conversion factor 1000; and the test portion in grams. Results are given to three significant figures.

7 The absolute difference between two independent determinations obtained under repeatability conditions does not exceed 23 % of their arithmetic mean.

8 With a 2 g test portion of cereals and cereal products, alcoholic drinks, soy sauce, vinegar, pastes and paste products, the limits of detection for deoxynivalenol, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol are 10 µg/kg and the limits of quantification 20 µg/kg. With a 5 g test portion of alcoholic drinks the limits of detection are 5 µg/kg and the limits of quantification 10 µg/kg.

12 Analytical procedure, second method

12.1 Sample preparation is as in 5.1.

12.2 Extraction. For cereals and cereal products 25 g of ground sample are weighed to 0.1 g into a 100 mL stoppered conical flask with 5 g of polyethylene glycol and 100 mL of water, mixed and sonicated or shaken 20 min, then filtered through glass fibre paper until the filtrate is clear, or centrifuged 10 min at 6 000 r/min, and centrifuged 5 min at 10 000 r/min to give filtrate A. For alcoholic drinks 20 g of sample are taken to 0.1 g with 1 g of polyethylene glycol, made to 25.0 mL with water and treated in the same way to give filtrate B. For soy sauce, vinegar, pastes and paste products 25 g are taken to 0.1 g with 5 g of polyethylene glycol, made to 100 mL with water and treated in the same way to give filtrate C.

12.3 Clean-up. The immunoaffinity column stored cold is brought to room temperature and allowed to drain. 2.0 mL of filtrate A, B or C is transferred to a glass syringe barrel connected to an air pressure pump and passed through the column at one drop per second until air enters the column. The column is washed with 5 mL of phosphate buffered saline and 5 mL of water at one to two drops per second until air enters it, all effluent is discarded and the column is drawn dry.

12.4 Elution. Exactly 2 mL of methanol is added and the column eluted at one drop per second, the whole eluate is collected in a tube and blown gently almost to dryness with nitrogen at 50 °C, 1.0 mL of initial mobile phase is added, the residue is dissolved by vortexing 30 s, and the solution is filtered through a 0.45 µm membrane into a sample vial.

12.5 Reference liquid chromatographic conditions: C18 column 150 mm long with an internal diameter of 4.6 mm and 5 µm packing, mobile phase methanol plus water (20 +