



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 5009.111-2003

Replacing GB/T 23503-2009; GB/T 5009.111-2003; SN/T 1571-2005

**Determination of deoxynivalenol in food -- High performance
liquid chromatographic method with immunoaffinity column
clean-up**

(English Translation)

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Foreword

This standard replaces GB/T 5009.111-2003 "cereal and cereal products deoxynivalenol bacteria enol determination", GB/T 23503-2009 "Food in deoxynivalenol bacteria enolase measurement immunoaffinity chromatography HPLC purification", SN/T 1571-2005 "Importers and deoxynivalenol in cereals in liquid chromatography test method." this standard compared with GB/T 5009.111-2003, the main changes are as follows.

- The name changed to the standard test "national food safety standards in food deoxynivalenol bacteria enolase and acetylated derivatives set";
- Increasing the scope of application of the method;
- Increased DETERMINATION deoxynivalenol bacteria enol acetylated derivatives;
- Increased isotope dilution liquid chromatography - tandem mass spectrometry;
- Increasing the solid-phase extraction (SPE) of the pre-treatment;
- Increased immunoaffinity column clean - high performance liquid chromatography;
- Increased commercialization immunoaffinity column evaluate technical parameters required.

National Food Safety Standard Foods deoxynivalenol bacteria enolase Determination and acetylated derivatives

1 Scope

This standard specifies the method for determination of deoxynivalenol in food fungus alcohol and acetylated derivatives.

The first method is by isotope dilution liquid chromatography - tandem mass spectrometry for cereal and cereal products, wine, soy sauce, vinegar, soy sauce and butter products off Oxygen snow rot Fusarium enol, 3-acetyl deoxynivalenol and 15-acetyl alcohol bacteria deoxynivalenol fungi alcohol measurement.

The second method is immunoaffinity chromatography and HPLC purification, suitable for grain and its products, wine, soy sauce, vinegar, soy sauce and butter products Determination of deoxynivalenol bacteria enolase.

The third method of thin layer chromatography assay, a fourth method of ELISA screening method for cereals and products deoxynivalenol bacteria Determination of alcohol.

The first method isotope dilution liquid chromatography - tandem mass spectrometry Principle 2 Sample of deoxynivalenol fungi alcohol, 3-acetyl deoxynivalenol and 15-acetyl alcohol bacteria deoxynivalenol fungi with water and alcohol A mixed solution of acetonitrile extraction, the supernatant after solid phase extraction columns or immuno-affinity column

purification, concentration, volume, and after filtration, high pressure liquid chromatography Spectral separation, tandem mass spectrometry, isotope internal standard.

3 Reagents and materials

Unless otherwise indicated, the methods used were of analytical grade reagents and water as a water GB/T 6682 regulations.

3.1 Reagents

3.1.1

acetonitrile (CH_3CN). chromatography.

3.1.2

Methanol (CH_3OH). chromatography.

3.1.3

n-hexane (C_6H_{14}).

3.1.4

Ammonia ($\text{NH}_3 \cdot \text{H}_2\text{O}$).

3.1.5

formic acid (HCOOH).

3.1.6

Nitrogen (N_2). purity $\geq 99.9\%$.

3.2 Reagent preparation

3.2.1

acetonitrile - water (8416). amount of 160mL of water was added to 840mL of acetonitrile and mix.

3.2.2

acetonitrile saturated solution of hexane. amount of n-hexane in 200mL 250mL separatory funnel, adding a small amount of acetonitrile, shake vigorously A few minutes, standing layer, discarding the lower acetonitrile layer, that is, too.

3.2.3

Methanol - water (595). amount of 5mL of methanol was added to 95mL of water, and mix.

3.2.4 0.01% aqueous ammonia solution. Take 100 μL aqueous ammonia was added to 1000mL of water, and mix (for ion source ESI- mode is used).

3.2.5 0.1% formic acid solution. Take 1mL formic acid is added to 1000mL of water, and mix (for ESI ion source mode is used).

3.3 Standard

3.3.1

deoxynivalenol bacteria enolase (DON, C₁₅H₂₀O₆, CAS number. 51481-10-8). purity $\geq 99\%$, or certified by the state and granted Standards certificate.

3.3.2 3-acetyl deoxynivalenol bacteria enolase (3-ADON, C₁₇H₂₂O₆, CAS number. 50722-38-8). purity $\geq 99\%$, or by the State Certified and standard substance certificate.

3.3.3 15- acetyl deoxynivalenol bacteria enolase (15-ADON, C₁₇H₂₂O₆, CAS number. 88337-96-6). purity $\geq 99\%$, or by State Hotels certified and reference material reference material certificate.

3.3.4 ¹³C¹⁵- deoxynivalenol fungi alcohol standard solution isotopes (¹³C-DON, ¹³C₁₅H₂₀O₆). 25μg/mL, the purity of $\geq 99\%$.

3.3.5 ¹³C¹⁷-3- acetyl - deoxynivalenol fungi alcohol standard solution isotopes (¹³C-3-ADON, ¹³C₁₇H₂₂O₆). 25μg/mL, purity $\geq 99\%$.

3.4 Standard Solution

3.4.1

Standard stock solution (100μg/mL). Weigh DON, 3-ADON and 15-ADON 1mg (accurate to 0.01mg), points Do not dissolved in acetonitrile and set the volume to 10mL. The solution was transferred to a vial, sealed and stored at -20 °C, valid for 1 year.

3.4.2

mixed standard working solution (10μg/mL). Imbibe 100μg/mL DON, 3-ADON and 15-ADON stock standard solution each 1.0mL in the same 10mL volumetric flask, add acetonitrile to volume. Sealed and stored at -20 °C, valid for six months.

3.4.3

Mixed isotope internal standard working solution (1μg/mL). Imbibe ¹³C¹⁵-DON and ¹³C¹⁷-3-ADON isotope internal standard (25μg/mL) each 1mL to 25mL same flask, add acetonitrile to volume. Sealed and stored at -20 °C, valid Six months.

3.4.4

standard series working solution. accurate Pipette the appropriate amount of mixed standard working solution and mixed isotope internal standard working solution with the initial mobile phase Formulated as 10ng/mL, 20ng/mL, 40ng/mL, 80ng/mL, 160ng/mL, 320ng/mL, mixed standard 640ng/mL of Series, in which the isotope internal standard at a concentration of 100ng/mL. Standard series solution at 4 °C preservation, valid 7d.