

ICS 67.050
CCS X 04



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

GB/T 5009.96-2003

Replaces GB/T 23502-2009; GB/T 25220-2010; GB/T 5009.96-2003; SN 0211-1993; SN/T 1746-2006; SN/T 1940-2007

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

(English Translation)

Issued on: December 23, 2016

Implemented on: June 23, 2017

**Issued by: National Health and Family Planning Commission of the
People's Republic of China, State Food and Drug Administration**

Table of Contents

Foreword
1 Scope
2 Principle
3 Reagents and materials
3.1 Reagents
3.2 Reagent preparation
3.3 Standards
3.4 Standard solution preparation
3.5 Materials
4 Instruments and equipment
5 Analysis steps
5.1 Preparation and extraction of samples
5.2 Sample purification
5.3 Elution
5.4 Specimen determination
6 Analysis of the results of the expression
7 Precision
8 Other
9 Principle
10 Reagents and materials
10.1 Reagents
10.2 Preparation of reagents
10.3 Standards
10.4 Standard solution preparation
11 Instruments and equipment
12 Analysis steps
12.1 Sample preparation
12.2 Sample extraction

Foreword

This standard replaces GB/T 23502-2009 "Determination of ochratoxin A in foods Immunoaffinity chromatography Purification High performance liquid chromatography Law ", GB/T 25220-2010" Determination of ochratoxin A in grain and oil inspection food by high performance liquid chromatography and fluorescence spectrophotometry " GB/T 5009.96-2003 "Determination of ochratoxin A in cereals and soybeans", SN/T 1746-2006 "Import and export of soybean, rapeseed and Method for the determination of ochratoxin A in edible vegetable oils ", SN/T 1940-2007" Determination of ochratoxin A in food for import and export Law "and SN0211-1993" Method for the detection of aflatoxin A in export grain ".

This standard compared with GB/T 23502-2009, the main changes are as follows.

- standard name changed to "food safety national standard food ochratoxin A determination";
- a third method of immunoaffinity chromatography for the purification of liquid chromatography - tandem mass spectrometry and the fourth method of enzyme - linked immunosorbent assay;
- increase the scope of application and optimize the extraction method;
- Removal of the immunoaffinity column chromatography purification fluorescence spectrophotometry.

National standards for food safety Determination of ochratoxin A in food

1 Scope

This standard specifies the determination of ochratoxin A in food.

The first method of this standard applies to cereals, oil and its products, alcohol, soy sauce, vinegar, sauce and sauce products, raisins, pepper tablets/powder ochrat Toxin A determination, the second method for corn, rice (brown rice), wheat, wheat flour, soybeans, coffee, wine ochratoxin A test the third method applies to corn, wheat and other food products, pepper and its products, beer and other alcohol, soy sauce and other products, raw coffee, cooked coffee Ochratoxin A determination, the fourth method for corn, wheat, barley, rice, soy and its products in the determination of ochratoxin A, the fifth method is applicable to the determination of ochratoxin A in wheat, maize and soybean.

First method immunoaffinity chromatography purification liquid chromatography

2 Principle

The ochratoxin A in the sample was extracted with the extract, purified by immunoaffinity column, and then analyzed by high performance liquid chromatography combined with fluorescence detector Determination of ochratoxin A content, external standard method quantitative.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and water is a primary water specified in GB/T 6682.

3.1 Reagents

3.1.1

Methanol (CH₃OH). Chromatographic Purification.

3.1.2

acetonitrile (CH₃CN). pure chromatography.

3.1.3

glacial acetic acid (C₂H₄O₂). pure chromatography.

3.1.4

Sodium chloride (NaCl).

3.1.5

Polyethylene glycol [HOCH₂ (CH₂O · CH₂)_nCH₂OH].

3.1.6

Tween 20 (C₅₈H₁₁₄O₂₆).

3.1.7

Sodium bicarbonate (NaHCO₃).

3.1.8

Potassium dihydrogen phosphate (KH₂PO₄).

3.1.9

concentrated hydrochloric acid (HCl).

3.1.10

nitrogen (N₂). purity ≥99.9%.

3.2 Reagent preparation

3.2.1

Extract I. Methanol - Water (80 20).

3.2.2

Extract II. Weigh 150.0 g of sodium chloride, 20.0 g of sodium bicarbonate dissolved in about 950 mL of water, add water to volume to 1 L.

3.2.3

Extract III. acetonitrile - water (60 40).

3.2.4

Washing solution. Weigh 25.0 g of sodium chloride, 5.0 g of sodium bicarbonate dissolved in about 950 mL of water, add water to volume to 1 L.

3.2.5

Mycotoxin Wash Buffer. Weigh 25.0 g of sodium chloride, 5.0 g of sodium bicarbonate dissolved in water, add 0.1 mL Tween 20, water Diluted to 1L.

3.2.6

phosphate buffer. Weigh 8.0 g of sodium chloride, 1.2 g of sodium hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate, 0.2 g of potassium chloride was dissolved in about 990 mL Water, with concentrated hydrochloric acid to adjust the pH to 7.0, diluted with water to 1L.

3.2.7

sodium bicarbonate solution (10g/L). Weigh 1.0g sodium bicarbonate, dissolved in water and diluted to 100mL.

3.2.8

Leaching buffer. 1.0 mL Tween 20 was added to 1000 mL of phosphate buffer.

3.3 Standards

Ochratoxin A (C₂₀H₁₈ClNO₆, CAS. 303-47-9), purity ≥99%. Or by the national certification and awarded the standard material certificate the standard material of the book.

3.4 Standard solution preparation

3.4.1

ochratoxin A standard stock solution. accurately weighed a certain amount of ochratoxin A standard, with methanol - acetonitrile (50 50) dissolved Solution, dubbed 0.1mg/mL