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

# 中华人民共和国国家标准

GB 5413.37-2010

# National food safety standard Determination of aflatoxin $M_1$ in milk and milk products

# 食品安全国家标准

# 乳和乳制品中黄曲霉毒素 $M_1$ 的测定

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**Issued by the Ministry of Health of P.R of China**

# Contents

# Foreword

Method I of this standard corresponding to ISO 14501: 2007 *Milk and milk powder-determination of aflatoxin $M_1$ content -clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography*, Method I of this standard is not equivalent to ISO 14501: 2007; Method II and III of this standard will replace GB/T 18980-2003; Method IV of this standard refers to NY/T 1664-2008 *Rapid determination for aflatoxin $M_1$ in milk-Double flow enzyme-linked immunosorbent assay.*

Appendix A and B of this standard is all informative.

# National food safety standard
# Determination of aflatoxin $M_1$ in milk and milk products

## 1. Scope

This standard specifies the method for determination of aflatoxin $M_1$ in milk and dairy products.

The first method in this standard applies to determination of aflatoxin $M_1$ in milk and dairy products; the second method applies to determination of aflatoxin $M_1$ in milk, milk powder, low fat milk, skimmed milk, low fat milk powder and skimmed milk powder; the third method applies to determination of aflatoxin $M_1$ in milk and milk powder; the fourth method applies to determination of aflatoxin $M_1$ in cow's milk and its products;

## 2. Normative References

The normative documents referenced in the text are indispensable to the application of this standard. For dated references, only the edition bearing such date applies to this standard. For undated references, the latest edition of the normative document referred to (including all the amendments) applies

## Method I Immunochromatography Clean-up Combined with Liquid Chromatography - Mass Spectrometry

## 3. Principles

Dissolve the test sample with mixed solution of water and organic solvent, extract it with ultrasound, centrifuge, take the supernatant which is then purified with an immuno-affinity column; dry the eluent with $N_2$, dilute to volume, filtrate with a microporous membrane, inject into a liquid chromatograph to separate, ionize it with an electrospray ion source, detect it with Multi Reaction Monitor (MRM), and then quantitate it with matrix plus external standard method.

## 4. Reagents and materials

Unless otherwise specified, all reagents used in this method are analytical reagents; water is the first grade water specified in GB/T6682.

**4.1** Formic acid: chromatographic pure.

**4.2** Acetonitrile: chromatographic pure.

**4.3** Petroleum benzine: boiling range is 30℃ to 60℃.

**4.4** Chloroform.

**4.5** Nitrogen gas: purity ≥99.9%.

**4.6** Standard sample: $M_1$: purity ≥ 98%.

**4.7** 20% acetonitrile water solution: add 100 mL acetonitrile into 400 mL water.

**4.8** 10% acetonitrile water solution: add 50 mL acetonitrile to 450 mL water.

**4.9** 0.1 % formic acid solution: pipette 1 mL formic acid and dilute it with water to 1000 mL.

**4.10** Acetonitrile /methanol solution (50+50): add 500 mL methanol into 500 mL acetonitrile.

**4.11** 0.5 mol/L 1 sodium hydroxide solution: weigh 2 g sodium hydroxide and dissolve with 1000 mL water.

**4.12** Blank matrix solution of the test sample

Weigh 8 test samples with the same matrix as the sample to be tested and without flavacin into a 100 mL beaker. Carry out the following operations according to 6.1 Extraction of Test Solution and 6.2 Purification procedures. Combine the purification solution from the 8 test samples, filter with a single-use filter with 0.22 μm microporous membrane (5.7), discard the first 0.5 mL filtrate, and take a small amount of the filtrate and detect it with liquid chromatography - mass spectrometry.

Acquire the chromatography - mass spectrum and compare it with figure A.2 in appendix A, there shouldn't be aflatoxin $M_1$ at corresponding retention time. Transfer the residual filtrate to a brown bottle, and store at a - 20°C refrigerator for preparation of the series of standard solutions.

**4.13** Standard stock solution of aflatoxin: weigh 0.10 mg standard substance M1 (accurate to 0.01 mg), dissolve and dilute to 10 mL with chloroform (4.4). The concentration of this standard solution is 0.01 mg.mL-1. Transfer the solution to a plastic bottle, store in a - 20 °C refrigerator for later use.

**4.14** A series of standard solutions:

Pipette 100 μL $M_1$ standard stock solution (4.14) to a 100 mL volumetric flask, blow chloroform with nitrogen gas to nearly dry, and dilute to volume with acetonitrile. Dilute $M_1$ standard stock solution (4.14) with blank matrix solution of the test sample (4.13) to a series of standard working solutions with concentrations with 0.5, 0.8, 1.0, 2.0, 4.0, 6.0 and 8.0 ng mL -1, and then dilute to 1 mL.

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