

ICS 67.020
CCS C 53



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

GB 29688-2013

**Determination of Chloramphenicol residues in milk by Liquid
Chromatography-tandem Mass Spectrometric method**

食品安全国家标准 牛奶中氯霉素残留量的测定 液相色谱-串联质谱法

(English Translation)

Issued on: September 16, 2013

Implemented on: January 1, 2014

**Issued by: Ministry of Agriculture of the People's Republic of China,
National Health and Family Planning Commission of the People's
Republic of China**

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1 Scope

This standard specifies chloramphenicol Residues in Milk sample preparation and liquid chromatography - tandem mass spectrometry.

This standard applies to milk the detection of chloramphenicol residues.

2 Normative references

The following documents for the application of this document is essential. For dated references, only applies to the version dated paper Pieces. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 6682-1992 specifications and test methods for water analysis laboratory Principle 3 Chloramphenicol residues in a sample, extracted with ethyl acetate, hexane degreasing, C18, purified by liquid chromatography - tandem mass spectrometry, internal standard method Quantitative.

4 Reagents and materials

The following reagents used, unless otherwise stated who were of analytical reagent; water as a water line with GB/T 6682 provisions.

Chloramphenicol 4.1 Standard. Content $\geq 97\%$.

4.2

Internal standard. Chloramphenicol deuterated standards. content $100\mu\text{g/mL}$ (as an internal standard stock standard solution).

4.3

Methanol. HPLC grade.

4.4

Acetonitrile. chromatographically pure.

4.5

ethyl acetate.

4.6

sodium chloride.

4.7

n-hexane.

4.8

C18 solid phase extraction column. $500\text{mg}/3\text{mL}$, or equivalent person.

4.9 4% sodium chloride solution. Take sodium chloride 4g, dissolve and dilute to 100mL with water.

4.10 $100\mu\text{g/mL}$ chloramphenicol standard stock solution. Weigh accurately chloramphenicol standard 10mg, in 100mL flask, dissolved in methanol and Diluted to the mark, formulated at a concentration of $100\mu\text{g/mL}$ standard stock solution of chloramphenicol. $-20\text{ }^{\circ}\text{C}$ below, valid for one year.

4.11 $100\mu\text{g/L}$ of chloramphenicol standard working solution. precise amount of $100\mu\text{g/mL}$ standard stock solution of chloramphenicol $100\mu\text{L}$, in 100mL Volumetric flask, dissolved in 50% acetonitrile and dilute to volume, formulated at a concentration of $100\mu\text{g/L}$ of the working standard solution. $2\text{ }^{\circ}\text{C} \sim 8\text{ }^{\circ}\text{C}$ preservation, effective 1 month.

4.12 $20\mu\text{g/L}$ of chloramphenicol deuterated standard working solution. the precise amount of a deuterated standard 20 L of chloramphenicol, in 1000mL flask, 50% acetonitrile dissolve and dilute to volume, formulated at a concentration of $20\mu\text{g/L}$ chloramphenicol deuterated standard working solution. $2\text{ }^{\circ}\text{C} \sim 8\text{ }^{\circ}\text{C}$ preservation, effective 3 months.

5 Apparatus

5.1

liquid chromatography - tandem mass spectrometry. ion source distribution.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

Vortex.

5.5

oscillator.

5.6

tissue homogenizer.

5.7

refrigerated centrifuge.

5.8

rotary evaporator.

5.9

centrifuge tube. 50mL.

5.10

heart-shaped bottle. 50mL.

5.11

SPE.

5.12

Nitrogen blowing instrument.

5.13

filter. 0.22μm.

Preparation and Storage of sample 6

6.1 Preparation of the sample

An appropriate amount of fresh or frozen or blank test milk, mixed, and homogenized.

--- the test sample taken after homogenization, as the feed try.

--- blank sample taken after homogenization, as a blank sample.

--- blank sample taken after homogenization, adding a suitable concentration of the standard working solution, is added as a blank sample.

Save 6.2 sample

Or less at -20 °C.

Determination Step 7

7.1 Extract

Take Sample $10\text{g} \pm 0.05\text{g}$, in 50mL centrifuge tubes, add deuterated internal standard working solution 250 L of chloramphenicol, together with ethyl acetate 20mL, Oscillation 15min, 6000r/min centrifugal 10min, the ethyl acetate layer was in heart-shaped flask. Coupled with 20mL ethyl acetate extraction was repeated a Times, the two extracts were combined heart-shaped flask in a water bath at 45 °C rotary evaporated to dryness. The residue was dissolved with 4% sodium chloride solution 5mL, plus 5mL hexane oscillations 1min, standing layered hexane was discarded. Coupled with n-hexane 5mL, extraction was repeated once. Take subnatant spare.

7.2 Purification

C18 column and washed with 5mL methanol and 5mL water activation, taking stock solution through the column, controlling the flow rate of 1 drop/(3s ~ 4s), 5mL water leaching Washed, drained, washed with methanol 5mL, collecting eluate, blown dry with nitrogen at 50 °C. With 50% acetonitrile and the residue was dissolved 1.0mL, vortex mixed Uniform, membrane filtration, for liquid chromatography - tandem mass spectrometry.

7.3 Preparation of standard curve

The precise amount of 100µg/L of chloramphenicol standard working solution and 20µg/L of chloramphenicol deuterated amount of internal standard working solution, dilute with mobile phase Release, formulated at a concentration of chloramphenicol 0.10,0.25,0.50,1.0,2.0,5.0µg/L, chloramphenicol deuterated concentration 5µg/L series of standard solutions Solution for liquid chromatography - tandem mass spectrometer. A characteristic ion mass chromatogram peak area for the vertical axis, the standard solution at abscissa, standard curve line. Seeking regression equation and correlation coefficient.