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

GB 29689-2013

**Determination of Thiamphenicol residues in milk by High
Performance Liquid Chromatographic method**

食品安全国家标准 牛奶中甲砒霉素残留量的测定 高效液相色谱法

(English Translation)

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Table of Contents

- 1 Scope
- 2 Normative references
- 4 Reagents and materials
- 5 Apparatus
- 6.1 Preparation of the sample
- 7.1 Extract
- 7.2 Purification
- 7.3 Preparation of standard curve
- 7.4 High Performance Liquid Chromatography
- 7.5 Blank test
- 9 Detection sensitivity, accuracy and precision
- 9.1 Sensitivity
- 9.2 Accuracy
- 9.3 Precision

1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method Thiamphenicol milk residues detected.

This standard applies to the detection of residual amount of milk thiamphenicol.

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 Water for analytical laboratory specifications and test methods Principle 3 the specimens remaining thiamphenicol, extracted with ethyl acetate, hexane degreasing, C18, purified by HPLC - UV determination, external standard the statutory amount.

4 Reagents and materials

The following reagents used, unless otherwise stated analytically pure, the water is in line with a water GB/T 6682 provisions.

4.1

thiamphenicol reference. content $\geq 99.0\%$.

4.2

ethyl acetate.

4.3

n-hexane.

4.4

Acetonitrile. chromatographically pure.

4.5

C18 solid phase extraction column. 200mg/3mL, or equivalent person.

4.6 1mg/mL thiamphenicol standard stock solution. Weigh accurately thiamphenicol reference substance 10mg, in 10mL volumetric flask, dissolved in acetonitrile and dilute to volume, formulated at a concentration of standard stock solution 1mg/mL of. 2 °C ~ 8 °C storage period of one month.

4.7 10 μ g/mL thiamphenicol standard working solution. precise amount of 1mg/mL standard stock solution thiamphenicol 1.0mL, in an amount of 100mL Flask, diluted with mobile phase, formulated at a concentration of 10 μ g/thiamphenicol mL of standard working solution. Now with the current.

5 Apparatus

5.1

HPLC. with UV detector.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

centrifuge.

5.5

SPE.

5.6

nitrogen blow instrument.

5.7

oscillating mixer.

5.8

heart-shaped bottle. 150mL.

5.9

rotary evaporator.

5.10

stopper plastic centrifuge tube. 50mL.

5.11

filter. 0.45 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

An appropriate amount of fresh or frozen or blank test milk and mix well.

--- 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 solution, is added as a blank sample.

Save 6.2 sample

Or less at -20 °C.

Determination Step 7

7.1 Extract

Sample Weigh $5g \pm 0.05g$, in 50mL centrifuge tubes, ethyl acetate was added 20mL, oscillation 10min, 4000r/min centrifugal 5min, the supernatant in the heart-shaped flask, the residue was added ethyl acetate 20mL, extraction was repeated once, the two extracts were combined, rotating at 45 °C Evaporated to near dryness, water was added to a 5mL flask heart-shaped, so that the ultrasonic 5min fully dissolved, transferred to 50mL centrifuge tubes. Add water to chicken 5mL After repeated dissolution vial heart go same centrifuge tube. 20mL hexane plus shaking 5min, 4000r/min centrifugal 2min, and the lower layer Liquid reserve.

7.2 Purification

C18 column eluted sequentially with acetonitrile and water 5mL 5mL activation. Take stock solution through the column, controlling the flow rate 1mL/min, squeezed. 5mL acetonitrile, Collecting eluate, dry nitrogen at 45 °C, the mobile phase the residue was dissolved 1.0mL, membrane filtration, for HPLC.

7.3 Preparation of standard curve

The precise amount of 10µg/mL thiamphenicol appropriate amount of working standard solution, diluted with mobile phase to prepare a concentration of 20,50,125,250 and 500µg/L of the series of standard solution for HPLC assay. To measure peak area for the vertical axis, the standard solution concentration corresponding to a cross Axis, the standard curve. Seeking regression equation and correlation coefficient.

7.4.1 Chromatographic conditions

7.4.1.1

Column. C18 (250mm x 4.6mm, particle size 5µm), or equivalent person.

7.4.1.2

Mobile phase. aqueous acetonitrile (20 80, by volume), filtered through a filter membrane prior to use ultrasound.

7.4.1.3

flow rate. 1.0mL/min.

7.4.1.4

Detection wavelength. 225nm.

7.4.1.5

Column temperature. 30 °C.

7.4.1.6

Injection volume. 20µL.

7.4.2 Assay

Take a sample solution and standard solutions corresponding, for single or multi-point calibration, by external standard method, the peak area is calculated. Standard solution and sample solution Thiamphenicol fluid response of the instrument to be within the linear range of detection. In the chromatographic conditions, the standard solution and the blank test tissue was added FIG HPLC sample solution are given in Appendix A.