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

**GB 29681-2013**

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

食品安全国家标准 牛奶中左旋咪唑残留量的测定 高效液相色谱法

**(English Translation)**

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### 1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method Residues in Milk levamisole detected.

This standard applies to milk Levamisole 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 Water for analytical laboratory specifications and test methods Principle 3 Levamisole remaining in a sample, extracted with ethyl acetate and carbonate buffer solution, C18 column purification, methanol, high performance liquid chromatography Spectroscopy, external standard.

## 4 Reagents and Materials

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

Levamisole hydrochloride 4.1 Standard. Content  $\geq 99.0\%$ .

### 4.2

methanol.

Anhydrous sodium bicarbonate 4.3. excellent pure.

### 4.4

sodium carbonate.

### 4.5

sodium dihydrogen phosphate.

### 4.6

concentrated ammonia.

### 4.7

diethylamine. excellent pure.

### 4.8

acetonitrile.

### 4.9

phosphoric acid.

### 4.10

hydrochloric acid.

### 4.11

C18 solid phase extraction column. 500mg/3mL.

4.12 10% phosphoric acid solution. Take phosphoric acid 70mL, dissolved and diluted with water to 1000mL.

4.13 3mol/L hydrochloric acid solution. Take 250mL of hydrochloric acid, dissolved and diluted with water to 1000mL.

### 4.14

saturated aqueous sodium bicarbonate. water 1000mL, anhydrous sodium bicarbonate was added to no dissolved, using now.

Saturated aqueous sodium carbonate 4.15. 500 mL water, was added to the sodium

carbonate is not dissolved, using now.

#### **4.16**

carbonate buffer (about pH = 9). Take a saturated aqueous solution of sodium bicarbonate 900mL, saturated aqueous sodium carbonate was added 100mL, and mix.

4.17 0.02mol/L sodium dihydrogen phosphate buffer solution diethylamine. Take 2.44 g of sodium dihydrogen phosphate, water was added 850mL dissolved, add diethylamine 3mL mixed with 10% phosphoric acid adjusted to pH 7.5, diluted with water to 1000mL.

4.18 1mg/mL levamisole standard stock solution. accurately weighed standard levamisole hydrochloride 10mg, in 10mL volumetric flask, washed with methanol Dissolved and diluted to the mark, formulated at a concentration of 1mg/mL of stock standard solution levamisole. 2 °C ~ 4 °C storage period of three months.

4.19 10µg/mL levamisole standard working solution. precise amount of 1mg/mL standard stock solution levamisole 1.0mL, in 100mL Volumetric flask, dilute to volume with methanol, formulated at a concentration of 10µg L levamisole standard working solution /. 2 °C ~ 4 °C, valid 3 months.

## **5 Apparatus**

### **5.1**

HPLC. with UV detector.

### **5.2**

Analytical balance. a sense of volume 0.00001g.

### **5.3**

Electronic balance. a sense of the amount of 0.01g.

### **5.4**

homogenizer.

### **5.5**

freezing high-speed centrifuge.

### **5.6**

electric heated water bath.

### **5.7**

vortex mixer.

## 5.8

eggplant-shaped bottle. 50mL.

## 5.9

centrifuge tube.

## 5.10

filter. 0.45μm.

Preparation and Storage of sample 6

### 6.1 Preparation of the sample

An appropriate amount of fresh or frozen or blank test milk, homogeneous mixing.

--- 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

Sample Weigh  $5g \pm 0.05g$ , a centrifuge tube, carbonate buffer was added 5mL, ethyl acetate was added 10mL, mix, 6000r/min Centrifugal 10min, the supernatant was eggplant-shaped flask, together with 10mL ethyl acetate and extracted once, twice combined supernatants rotating at 50 °C water bath Evaporated to dryness, the residue dissolved in carbonate buffer was 5mL standby.

### 7.2 Purification

3mL C18 column successively with water, methanol and 3mL 3mL activated carbonate buffer, taking stock solution through the column, 3mL water rinsed, dried A Alcohol 5mL, collecting eluate, dry nitrogen at 50 deg.] C water bath, the residue was dissolved in mobile phase was 1.0mL, membrane filtration, high performance liquid supply Phase Chromatography.