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PEOPLE'S REPUBLIC OF CHINA**

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

食品安全国家标准 牛奶中喹诺酮类药物多残留的测定 高效液相色谱法

(English Translation)

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1 Scope

This method provides a sample preparation and high performance liquid chromatographic method Residues in Milk quinolone drug testing.

This method is applicable to milk ciprofloxacin, danofloxacin residues, enrofloxacin, sarafloxacin and difloxacin single or multiple drugs Detection.

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 Quinolones in a sample extracted with acetonitrile, and rotary evaporated to near dryness, dissolved in mobile phase. High Performance Liquid Chromatography - Fluorescence Fixed, external standard.

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.

4.1

danofloxacin, enrofloxacin, ciprofloxacin hydrochloride, hydrochloric acid sarafloxacin stars and dihydrochloride difloxacin reference. content $\geq 99.0\%$.

4.2

phosphoric acid.

4.3

sodium hydroxide.

4.4

Acetonitrile. chromatographically pure.

4.5

triethylamine.

A saturated solution of sodium hydroxide 4.6. take sodium hydroxide appropriate amount, add water and shake to make into a saturated solution, after cooling, the setting of polyethylene plastic bottle, allowed to stand, clarify.

4.7 5mol/L sodium hydroxide solution. saturated solution of sodium hydroxide to take 28mL,

dissolve and dilute to 100mL with water.

4.8 0.03mol/L sodium hydroxide solution. Take 5mol/L sodium hydroxide solution 0.6mL, dissolve and dilute to 100mL with water.

4.9 0.05mol/L phosphoric acid solution triethylamine. phosphoric take 3.4mL, dissolved and diluted with water to 1000mL. Adjusted with triethylamine pH to 2.4.

4.10

quinolones mixed standard stock solution. Precision Weigh danofloxacin reference substance 10mg, enrofloxacin, ciprofloxacin, sarafloxacin and difloxacin reference substance 50mg, in 50mL volumetric flask, dissolve and dilute to volume with 0.03mol/L sodium hydroxide solution, formulated Danofloxacin at a concentration of 0.2mg/mL, ciprofloxacin, enrofloxacin, sarafloxacin and difloxacin concentration of 1mg/mL quinolones Standard mixing the drug stock solution. 2 °C ~ 8 °C storage period of three months.

4.11

quinolones working standard solutions. quinolones precise amount of mixed standard stock solution 1.0mL, in 100mL flask in, diluted with mobile phase, danofloxacin formulated at a concentration of 2µg/mL, ciprofloxacin, enrofloxacin, sarafloxacin and difloxacin concentration 10µg/mL quinolones working standard solutions. 2 °C ~ 8 °C, valid for 1 week.

5 Equipment

5.1

HPLC. with a fluorescence detector.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

oscillator.

5.5

centrifuge.

5.6

PTFE tube. 50mL.

5.7

heart-shaped bottle. 25mL.

5.8

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 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 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 $2g \pm 0.05g$, in 50mL centrifuge tubes, add 100 L phosphoric acid, 4 mL of acetonitrile, vortexing, shaking speed 5min, 10000r/min centrifugal 10min, supernatant to another centrifuge tube, add n-hexane 5mL, scroll 1min, standing, and the lower layer in the serum 25mL heart-shaped bottle. 4 mL acetonitrile was added to the residue, extraction was repeated once, the supernatant was assigned with a n-hexane, the two extracts were combined, Rotary evaporator at 50 deg.] C to a yellow oil droplets of only the remaining easy to dryness. The residue was dissolved with 1.0mL flow membrane filtration for HPLC Determination chromatography.

7.2 Preparation of standard curve

The precise amount of working standard solutions quinolones amount, diluted with mobile phase to prepare a concentration of ciprofloxacin, enrofloxacin, salad Gatifloxacin and difloxacin as 5,10,50,100,300 and 500μg/L, the concentration of danofloxacin 1,2,10,20,60 and 100μg/L in series Standard solution for HPLC assay. To measure peak area for the vertical axis, corresponding to the concentration of standard solution of abscissa, the standard curve line. Seeking regression equation and correlation coefficient.