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

GB 29698-2013

**Determination of 17 β -Estradiol, Estriol and Ethinylestradiol
Residues in Milk and Milk Products by Gas
Chromatography-Mass Spectrometric method**

食品安全国家标准 奶及奶制品中17 β -雌二醇、雌三醇、炔雌醇多残留的测定 气
相色谱-质谱法

(English Translation)

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1 Scope

This standard specifies the GC sample preparation and drug residues in milk and dairy products in an amount detectable estrogen - mass spectrometry.

This standard applies to milk and milk samples detected 17 β - estradiol, estriol, ethinylestradiol single or a plurality of drug 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 Estrogen remaining in a sample, a solvent mixture of ethyl acetate and acetonitrile extraction, solid-phase extraction column, derivatized silylating agent, the mold plasma Formula Gas Chromatography - Mass Spectrometry, external standard.

4 Reagents and materials

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

4.1 17 β - estradiol, estriol, ethinyl estradiol. content $\geq 98.0\%$.

4.2

disulfide erythritol.

4.3

N- methyl trimethylsilyl trifluoroacetyl ammonia.

4.4

trimethylsilane.

4.5

acetonitrile.

4.6

ethyl acetate.

4.7

methanol.

4.8

toluene.

4.9

n-hexane.

4.10

hydrochloric acid.

4.11

sodium hydroxide.

4.12

C18 solid phase extraction column. LC-C18, 500mg/3mL, or equivalent person.

4.13

silica gel solid phase extraction column. LC-Si, 500mg/3mL, or equivalent person.

4.14 95% hexane in ethyl acetate. n-hexane to take 95mL, dissolved with ethyl acetate and dilute to 100mL.

4.15

hexane 70% ethyl acetate. hexane to take 70mL, dissolved with ethyl acetate and dilute to 100mL.

4.16 1mol/L sodium hydroxide solution. take sodium hydroxide 40g, dissolved and diluted with water to 1000mL.

4.17 5mol/L hydrochloric acid solution. Take concentrated hydrochloric acid 48mL, dissolve and dilute to 100mL with water.

4.18

derivatizing agent. take dithio erythritol 0.01g, dissolved in N- methyl trimethylsilyl trifluoroacetyl ammonia (MSTFA) 5mL, in Adding trimethylsilyl subsurface 10 L, mix, 2 °C ~ 8 °C overnight, dark moisture sealed. Derivatizing agent should be colorless, if Brown-red color change occurs, etc., indicate reagent failure.

4.19 1mg/mL17beta- estradiol, estriol, ethinylestradiol standard stock solution. Weigh accurately 17beta- estradiol, estriol, ethinyl estradiol standard each 10mg, respectively 10mL volumetric flask, dissolved in methanol and diluted to the mark, dubbed the concentration of the standard stock solution 1mg/mL of.

-20 °C below, valid for six months.

4.20 10mg/L17beta- estradiol, estriol, ethinylestradiol working standard solutions. precise amount of 1mg/mL17beta- estradiol, estriol Ethinyl estradiol and 1.0 mL of each standard stock solution, to a 100mL volumetric flask, dilute to the mark with methanol, formulated at a concentration of 10mg/L in a mixed Working standard solution, 2 °C ~ 8 °C storage period of one month.

5 Apparatus

5.1

Gas chromatography - mass spectrometry. EI source.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

Nitrogen blowing instrument.

5.5

SPE.

5.6

homogenizer.

5.7

vortex mixer.

5.8

centrifuge.

5.9

oven.

5.10

pH meter.

5.11

rotary concentrator.

5.12

filter. 0.22 μ m.

6.1 Preparation of the sample

An appropriate amount of fresh or frozen blank or test sample, 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

Save 2 °C ~ 8 °C.

Determination Step 7

7.1 Preparation of standard curve

The precise amount of 10mg/L working standard solutions amount, diluted with methanol, and formulated to a concentration of 10,50,100,200,500 1000 μ g/L series of working standard solution, blown dry with nitrogen at 40 °C water bath, treated according to the derivatization step, process gas chromatography - mass spectrometry. With Measured peak