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

GB 29683-2013

**Determination of Paracetamol residues in animal derived food
by High Performance Liquid Chromatographic method**

食品安全国家标准 动物性食品中对乙酰氨基酚残留量的测定 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 for animal food in an amount of acetaminophen residue detection.

This standard applies to pigs, cattle and sheep muscle, liver and kidney tissues acetyl detected residual amount of acetaminophen.

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 Remaining in a sample of acetaminophen, extracted with ethyl acetate, an HLB purification column, eluting with methanol, dried with nitrogen, dissolved in mobile phase, and efficient Liquid chromatography, external standard.

4 Reagents and materials

The following reagents used, unless otherwise stated who were of analytical grade; water line with an aqueous GB/T 6682 provisions.

4.1

pairs of acetaminophen standard. content $\geq 99\%$.

4.2

Methanol. HPLC grade.

4.3

ethyl acetate.

4.4

n-hexane.

4.5

HLB solid phase extraction column. 60mg/3mL, or equivalent person.

4.6 5% methanol aqueous solution. Methanol 5mL, dissolved and diluted to 100mL with water.

4.7 0.05mol/L ammonium acetate solution. Take 3.15 g of ammonium acetate, dissolved and diluted to 1000mL with water, membrane filtration.

4.8 1mg/mL acetaminophen standard stock solution. Weigh accurately acetaminophen 10mg, in 10mL volumetric flask, dissolved with methanol Solution and dilute to volume, formulated at a concentration of 1mg/mL of acetaminophen standard stock solution. -20 °C the following, valid for 3 months.

4.9 10 μ g/mL working solution of paracetamol standard. precise amount of 1mg/mL acetaminophen standard stock solution 1.0mL, in 100mL flask, dissolved in mobile phase and dilute to volume, formulated at a concentration of 10 μ g/working fluid acetaminophen standard mL.

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

freezing high-speed centrifuge.

5.5

homogenizer.

5.6

vortex mixer.

5.7

circulating water pump.

5.8

stoppered centrifuge tube. 50mL.

5.9

filter. 0.45 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or frozen blank or test tissue, fascia removed, minced, 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

Sample Weigh 5g $\pm$ 0.05g, centrifuge tube with stopper, ethyl acetate was added 20mL vortex 2min, 6000r/min centrifugal 15min, the supernatant, in another centrifuge tube, the residue was added ethyl acetate 10mL, extraction was repeated once, the two supernatants were combined, 6000r/min from Heart 10min, the supernatant was evaporated to dryness in a water bath 45 °C, the residue was dissolved 3mL methanol, n-hexane was added 5mL, mixed, allowed to stand points Layer, hexane layer was discarded, the lower layer was

taken, dried with nitrogen at 45 °C water bath, dissolve the residue was washed with water 6mL, 10000r/min centrifugal 10min, the supernatant was set aside.

7.2 Purification

HLB column eluted sequentially with methanol and 3mL 3mL water activation, taking stock solution through the column, controlling the flow rate of less than 2mL/min. 3mL water 5% methanol and 3mL rinse, 4 mL methanol, the eluate was collected, dried at 40 °C ~ 45 °C water bath nitrogen gas, the mobile phase 1.0mL the residue was dissolved, 10000r/min centrifuge 5min, the supernatant for HPLC.

7.3 Preparation of standard curve

The precise amount of 10µg/mL working solution of paracetamol standard amount, diluted with mobile phase, concentration of formulated 10,25,50,100, 200,400,800 and 1600µg/L of the series of standard solution for HPLC assay. Peak area was measured to the vertical axis, corresponding to the standard Standard solution concentration as the abscissa, the standard curve. Seeking regression equation and correlation coefficient.

7.4.1 LC Conditions

7.4.1.1

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

7.4.1.2

Mobile phase. methanol 0.05mol/L ammonium acetate solution (20 80, volume ratio).

7.4.1.3

flow rate. 1.0mL/min.

7.4.1.4

UV detection wavelength. 250nm.

7.4.1.5

Injection volume. 50µL.

7.4.1.6

Column temperature. 30 °C.

7.4.2 Assay

Take a sample solution and standard solutions corresponding, for single or multi-point calibration, external standard method with peak area calculations. Standard solution and sample solution Acetaminophen in response shall be within the linear range of the detection instrument. In the chromatographic conditions, sample was added standard