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

GB 29709-2013

**Determination of Azaperone and metabolite in animal derived
food by High Performance Liquid Chromatographic method**

食品安全国家标准 动物性食品中氮哌酮及其代谢物残留量的测定 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 in animal food azapirone of nitrogen and residual piperidine alcohol metabolite detected.

This standard applies to porcine muscle, sebum, liver and kidney tissues and the detection of residual amount of azapirone nitrogen piperidin alcohol metabolites.

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.

Specifications and test methods for laboratory water GB/T 6682 analysis.

Principle 3

In a sample and the residual azapirone metabolite, extracted with acetonitrile, hexane degreasing, MCX column purification, HPLC, external standard the statutory amount.

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

azapirone, piperazine nitrogen alcohols standard. content $\geq 98\%$.

4.2

Acetonitrile. chromatographically pure.

4.3

n-hexane.

4.4

concentrated ammonia.

4.5

methanol.

4.6

ammonium acetate.

4.7

formic acid.

4.8

MCX SPE. 60mg/3mL, or equivalent person.

4.9 2% aqueous formic acid. formic acid take 2mL, dissolve and dilute to 100mL with water.

4.10 5% ammoniated methanol. aqueous ammonia Take 5mL, dissolved in methanol and diluted to 100mL.

4.11 5mmol/L ammonium acetate solution. Take ammonium acetate 0.385g, dissolved in water and diluted to 1000mL, membrane filtration.

4.12 1mg/mL standard stock solution. Weigh accurately azapirone piperazine nitrogen and alcohol in each standard 10mg, respectively 10mL volumetric flask, washed with methanol Dissolved and diluted to the mark, dubbed the concentration of the standard stock solution 1mg/mL of. The following -20 °C dark preservation, valid for three months.

4.13 1 μ g/mL working standard solutions. precise amount of each 1mg/mL standard stock solution azapirone piperidine nitrogen and the alcohol of each stock standard solution 0.1 mL, to 100mL volumetric flask, dilute to volume with methanol to prepare a working standard solutions at a concentration of 1 μ g/mL of. At -20 °C Stored in the dark period of

three months.

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

nitrogen blow device.

5.6

vortex mixer.

5.7

SPE.

5.8

ultrasonic cleaning machine.

5.9

homogenizer.

5.10

centrifuge tube.

5.11

filter. 0.22 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or frozen tissue white, the 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 $5\text{g} \pm 0.05\text{g}$, a centrifuge tube, acetonitrile was added 10mL, vortexed 1min, ultrasonic 2min, 5000r/min centrifugal 5min, the supernatant, the residue was added 10mL of acetonitrile extraction was repeated once, the two supernatants were combined. 40 °C rotary evaporated to dryness in a water bath. use 2% formic acid were dissolved 5mL aqueous residue was added 4 mL of n-hexane, mixed, 5000r/min centrifugal 5min, n-hexane layer was discarded, the standby.

7.2 Purification

MCX cartridge 3mL successively with methanol, water and 2% aqueous formic acid 3mL 3mL activation, taking stock solution through the column, the flow rate is less than the control 1mL/min, with 2% aqueous formic acid and methanol 3mL 3mL rinsed, drained, washed with 5% ammoniated methanol 4mL. The eluent was collected in 40 °C nitrogen blow, the residue was dissolved in mobile phase was 1.0mL, Vortex for HPLC.

7.3 Preparation of standard curve

The precise amount of $1\mu\text{g/mL}$ working standard solutions amount, diluted with methanol, and formulated to a concentration of 10,20,50,100,200,500 1000 $\mu\text{g/L}$ mixed standard solution series. Each concentration was repeated 3 times for HPLC, to measure the average peak area obtained for the vertical Coordinates, the corresponding standard concentration as the abscissa, 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 $5\mu\text{m}$), or equivalent person.

7.4.1.2

mobile phase. acetonitrile 5mmol/L ammonium acetate solution (50 50, volume ratio).

7.4.1.3

flow rate. 1mL/min.