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

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

食品安全国家标准 动物性食品中常山酮残留量的测定 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 Residues of halofuginone.

Detection of the residual amount of halofuginone This standard applies to the chicken muscle and liver tissues.

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 Halofuginone remaining in a sample, by trypsin digestion, extracted with ethyl acetate, liquid-liquid extraction, an HLB, purified by high performance liquid chromatography - Purple Determination outside, 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

halofuginone hydrobromide reference. content $\geq 99.6\%$.

4.2

Methanol. HPLC grade.

4.3

ethyl acetate. chromatography.

4.4

Acetonitrile. chromatographically pure.

Anhydrous sodium carbonate 4.5.

4.6

Trypsin. 1000U/mg.

4.7

ammonium acetate.

4.8

acetic acid.

4.9

toluene.

4.10

HLB solid phase extraction column. 500mg/6mL, or equivalent person.

4.11 0.25mol/L ammonium acetate buffer. Take 19.27 g ammonium acetate, 30mL acetic acid was added, dissolved and diluted to 1000mL with water, with ethyl Acid adjusted to pH 4.3.

4.12 0.125mol/L ammonium acetate buffer. Take 0.25mol/L ammonium acetate buffer 500mL, dissolved in water and diluted to 1000mL.

4.13 10% sodium carbonate solution. anhydrous sodium carbonate 10g, dissolve and dilute to 100mL with water.

4.14

mobile phase. acetonitrile take 250mL, 0.25mol/L ammonium acetate buffer 150mL and 600 mL of water, mixing, pH was adjusted with glacial acetic acid to 4.3, membrane filtration, now with the current.

4.15 200µg/mL halofuginone standard stock solution. Weigh accurately halofuginone hydrobromide standard 10mg, brown in 50mL flask with 0.25mol/L ammonium acetate buffer solution and diluted to the mark, formulated at a concentration of 200µg/mL standard stock solution of halofuginone. seal, Wrapped in aluminum foil, 2 °C ~ 8 °C from light, valid for 6 months.

4.16 10µg/mL standard stock solution halofuginone. precise amount of 200µg/mL standard stock solution 0.5mL halofuginone brown amount in 10mL Flask, dissolve and dilute to volume with water, is formulated at a concentration of 10µg/mL standard stock solution halofuginone. Sealed, wrapped in aluminum foil, 2 °C ~ 8 °C dark preservation, valid for six 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

centrifuge.

5.5

homogenizer.

5.6

a vortex.

5.7

rotary evaporator.

5.8

Nitrogen blowing instrument.

5.9

SPE.

5.10

constant temperature water bath.

5.11

filter. 0.45 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or thawed take appropriate blank or test tissue, 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

Weigh sample $4\text{g} \pm 0.04\text{g}$, in 100mL centrifuge tube, add trypsin 50mg, water was added 2 mL (liver) or 10mL water (Muscle tissue), vortex 1min, washed with 10% sodium carbonate solution to adjust the pH to 8.0, enzymatic 3h water bath at 40 °C. Removed, returned to room temperature, was added 10% sodium carbonate solution 2mL, vortexed 1min, combined with ethyl acetate 20mL, whirl 10s, 3min allowed to stand in an ice water bath, immediately 2000r/min centrifugal 2min, 100mL supernatant was transferred to a separatory funnel. Layer liquid extracted with ethyl acetate 20mL repeated once, Two supernatant were combined in the same separatory funnel, add 0.125mol/L ammonium acetate solution 10mL, shake for 1min, standing 2min, the collection the aqueous phase layer. Coupled 0.125mol/L ammonium acetate solution 10mL, extraction was repeated once, the two aqueous phases were combined, transferred to heart-shaped flask, at 38 °C Rotary evaporator, the residue divisible ethyl acetate and set aside.

7.2 Purification

HLB 3mL column successively with methanol, water and 3mL 0.125mol/L ammonium acetate 3mL activation, taking stock solution through the column, the flow rate control