

ICS 67.020
CCS C 53



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

GB 29690-2013

**Determination of marker residues of Nicarbazin in animal
derived food by Liquid Chromatography-tandem Mass
Spectrometric method**

食品安全国家标准 动物性食品中尼卡巴嗪残留标志物残留量的测定 液相色谱-串
联质谱法

(English Translation)

Issued on: September 16, 2013

Implemented on: January 1, 2014

**Issued by: Ministry of Agriculture of the People's Republic of China,
National Health and Family Planning Commission of the People's
Republic of China**

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1 Scope

This standard specifies the animal food residue nicarbazin marker 4,4-dinitrophenyl biuret Residues sample preparation and liquid chromatography Spectrum - tandem mass spectrometry.

This standard applies to chicken eggs and muscle tissue markers nicarbazin residues were detected 4,4'-dinitro diphenyl urea 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 the sample of residual 4,4-dinitrobenzene biuret, extracted with acetonitrile, hexane degreasing, extracted with 75% aqueous methanol solution, liquid chromatography - tandem Mass spectrometry, as internal 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 4,4-dinitro-stilbene urea reference. content of $\geq 98.0\%$.

4.2 4,4-dinitro-stilbene urea -D8 reference. content of $\geq 98.0\%$.

4.3

Acetonitrile. chromatographically pure.

4.4

Methanol. HPLC grade.

4.5

over anhydrous sodium sulfate.

4.6

n-hexane.

4.7

ammonium acetate.

4.8

dimethylformamide.

4.9 0.1mol/L ammonium acetate solution. Take ammonium acetate 1.93g, dissolve and dilute to 250mL with water.

4.10 75% aqueous methanol. Methanol 75mL, dissolved and diluted to 100mL with water.

4.11 75% saturated aqueous methanol n-hexane. Take 75% aqueous methanol 100mL, 100mL of n-hexane was added, shake and let stand stratification, the upper liquid.

4.12 1mg/mL 4,4'- dinitro diphenyl ureas are standard stock solution. Weigh accurately 4,4'-dinitro-stilbene urea reference substance 10mg, in 10mL volumetric flask, dissolved with dimethyl formamide and diluted to the mark, formulated at a concentration of 4,4-dinitro 1mg/mL standard were diphenylurea Quasi stock solution. 2 °C ~ 8 °C storage period of three months.

4.13 10 μ g/mL 4,4'- urea-dinitro-stilbene standard working solution. precise amount of 1mg/mL 4,4'- dinitro diphenyl ureas are standard stock 1.0 mL solution, in 100mL flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 4,4-dinitro-10 μ g/mL were two Phenylurea standard working solution. -18 °C, valid for three months.

4.14 1mg/mL 4,4'-dinitro diphenyl urea -D8 are standard stock solution. Weigh accurately 4,4'-dinitro-stilbene urea reference -D8 10mg, in 10mL volumetric flask, dissolve and dilute to volume with dimethylformamide, prepared at a concentration of 4,4-dinitro 1mg/mL of Diphenylurea -D8 are standard stock solution. 2 °C ~ 8 °C storage period of three months.

4.15 10µg/mL 4,4'-dinitro-stilbene urea -D8 standard working solution. precise amount of 1mg/mL 4,4'-dinitro-stilbene urea -D8 1.0 mL standard stock solution, to a 100mL flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 10µg/mL 4,4'-bis Nitro stilbene urea -D8 working standard solution. -18 °C, valid for three months.

5 Apparatus

5.1

liquid chromatography - tandem mass spectrometry. ion source distribution.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

a vortex.

5.5

centrifuge.

5.6

Nitrogen blowing instrument.

5.7

centrifuge tube. 50mL.

5.8

Membrane. 0.2µm.

Preparation and Storage of sample 6

6.1.1 Eggs

An appropriate amount of fresh or frozen egg white or test, peeled, 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.

6.1.2 Chicken muscle

Fresh or frozen 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 Extraction and purification

Sample Weigh $2g \pm 0.02g$, in 50mL centrifuge tubes, was added $10\mu g/mL$ 4,4'-dinitro-stilbene working standard solution of urea suitable -D8 Amount of anhydrous sodium sulfate 2g, 8 mL of acetonitrile, vortexed 0.5min, ultrasonic 5min, 5000r/min centrifugal 10min, supernatant at 40 °C dry nitrogen, was added 75% aqueous methanol saturated with hexane 1mL, scroll 10s, plus 1.0 mL of 75% aqueous methanol, sufficient Vortexed, allowed to stand in a water bath at 40 °C 5min, 2000r/min centrifugal 5min, the supernatant and the lower layer, membrane filtration, for liquid chromatography - tandem Mass Spectrometry.

7.2 Preparation of standard curve

The precise amount of $10\mu g/mL$ 4,4'-dinitro-stilbene working standard solutions of urea and $10\mu g/mL$ 4,4'-dinitro-stilbene standard urea -D8 Working fluid amount, diluted with 75% aqueous methanol solution, were formulated as 4,4'-dinitro diphenyl urea concentration -D8 are 100ng/mL and 4,4'-Dinitro-stilbene 2,10,20,50,200 and urea concentration is 500ng/mL series control solution for liquid chromatography - tandem mass spectrometry.

A characteristic ion mass chromatogram peak area for the vertical axis, the standard solution at abscissa, the standard curve. Seeking regression equation and correlation coefficient.

7.3.1 LC Conditions

7.3.1.1

Column. C18 (150mm x 2.1mm, particle size $5\mu m$), or equivalent person.