

ICS 67.020
CCS C 53



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

GB 29691-2013

**Determination of Nicarbazin residues in edible tissues of
chicken by High Performance Liquid Chromatographic method**

食品安全国家标准 鸡可食性组织中尼卡巴嗪残留量的测定 高效液相色谱法

(English Translation)

Issued on: October 17, 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**

Table of Contents

- 1 Scope
- 2 Normative references
- 4 Reagents and materials
- 5 Apparatus
- 6.1 Preparation of the sample
- 7.1 Extract
- 7.2 Purification
- 7.3 Preparation of standard curve
- 7.4 Determination
- 7.5 Blank test
- 9 Sensitivity detection method, accuracy and precision
- 9.1 Sensitivity
- 9.2 Accuracy
- 9.3 Precision

1 Scope

This standard specifies the edible chicken tissues nicarbazin identified residue of 4,4'-stilbene-nitro urea Residues high and sample preparation Performance liquid chromatographic method.

This standard applies to chicken muscle, liver and kidney tissues identified nicarbazin residue nitro stilbene 4,4' urea Residues Determination.

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 4,4'-dinitro diphenyl urea were extracted with acetonitrile, hexane degreasing, C18 column purification eluted aqueous acetonitrile, efficient Liquid chromatography, external standard.

4 Reagents and materials

The following reagents used, unless otherwise indicated, are analytical reagents, water is in line with a water GB/T 6682 provisions.

4.1 4,4-dinitro-stilbene urea reference. content of $\geq 99\%$.

4.2

N, N- dimethylformamide.

4.3

Acetonitrile. chromatographically pure.

4.4

Methanol. HPLC grade.

4.5

hexane. chromatography.

4.6

n-propanol.

4.7

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

4.8

Eluent. acetonitrile take 70mL, dissolve and dilute to 100mL with water.

4.9 1mg/mL 4,4'- dinitro diphenyl ureas are standard stock solution. Weigh accurately 4,4'-dinitro-stilbene urea reference 25mg, in 25mL volumetric flask, dissolve and dilute to volume with dimethylformamide, prepared at a concentration of 4,4-dinitro 1mg/mL are two Phenylurea standard stock solution. $-20\text{ }^{\circ}\text{C}$ below, valid for six months.

4.10 100 $\mu\text{g/mL}$ 4,4'- urea-dinitro-stilbene standard working solution. precise amount of 1mg/mL 4,4'- standard urea-dinitro-stilbene reservoir 1.0 mL was prepared, in 10mL volumetric flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 4,4-dinitro 100 $\mu\text{g/mL}$ of Stilbene urea working standard solution. The following $2\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$, valid for 1 week.

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

homogenizer.

5.5

rotary evaporator.

5.6

vortex mixer.

5.7

ultrasonic cleaning.

5.8

centrifuge.

5.9

heart-shaped bottle. 50mL.

5.10

stoppered centrifuge tube. 10mL, 50mL.

5.11

Membrane. organic phase, 0.45μm.

Preparation of sample material 6 and stored

6.1 Preparation of the sample

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 Extract

Sample Weigh $2.0\text{g} \pm 0.02\text{g}$, in 50mL centrifuge tube, acetonitrile was added 10mL, ultrasonic 5min, 4000r/min centrifugal 12min, the supernatant, heart-shaped flask in 50mL. The residue in acetonitrile was added 10mL, extraction was repeated once, the two supernatants were combined, added 3 mL of n-propanol, 60 °C rotary evaporated to near dryness. Was added 0.5 mL of acetonitrile, hexane 1mL, vortexed 3min, dissolved, to move with a plug 10mL centrifuge tube. With B Nitriles hexane 1mL 0.5mL repeated vortexing and once dissolved. The combined solution twice to 10mL stoppered centrifuge tube, was added n-hexane 2mL, vortex mixed 3min, 4000r/min centrifugal 5min, discarded the upper n-hexane solution. Coupled with hexane 2mL, extraction was repeated once, 4000r/min centrifugal 5min, the lower layer was taken and set aside.

7.2 Purification

C18 column with acetonitrile 10mL activated, taking stock solution through the column, the natural flow of dry, filtrate was collected. 4mL with the eluent, the eluate the filtrate and eluate were combined, rotary evaporated at 60 deg.] C to near dryness, the residue was dissolved in mobile phase was 2.0mL, membrane filtration, high performance liquid chromatography for Determination.

7.3 Preparation of standard curve

The precise amount of $100\mu\text{g/mL}$ 4,4'- dinitro-stilbene amount of urea working standard solution, diluted with mobile phase, formulated at a concentration of 31.25, 62.5, 125, 250, 500, 1000, 2000, 4000 and $8000\mu\text{g/L}$ of the series of standard solution for HPLC assay. With measured Peak area for the vertical axis, corresponding to the concentration of standard solution as 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. aqueous acetonitrile (58:42, volume ratio).

7.4.1.3

flow rate. 1.0mL/min.

7.4.1.4

Detector. UV detector.