

ICS 67.020
CCS C 53



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

GB 29699-2013

**Determination of Clopidol residues in chicken muscle by Gas
Chromatography-mass Spectrometric method**

食品安全国家标准 鸡肌肉组织中氯羟吡啶残留量的测定 气相色谱-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**

Table of Contents

1	Scope
2	Normative references
4	Reagents and Materials
5	Instruments and equipment
6.1	Preparation of the sample
7.1	Preparation of matrix-matched standard curve
7.2	Extract
7.3	Purification
7.5	Determination
7.6	Blank test
9	Detection sensitivity, accuracy and precision
9.1	Sensitivity
9.2	Accuracy
9.3	Precision

1 Scope

This standard specifies the sample preparation and gas chromatography chicken muscle tissue clopidol of Residues - mass spectrometry.

This standard is applicable to the determination of chicken muscle tissue pyridine chlorine hydroxyalkyl 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 clopidol, extracted with acetonitrile, basic alumina column purification, N, O- bis (trimethylsilyl) trifluoroacetamide and three Chlorosilane derivatization gas chromatography - mass spectrometry, 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.

Clopidol Reference Sample 4.1. content of $\geq 99\%$.

Anhydrous sodium sulfate 4.2. Before use in the muffle furnace at 500 °C 5h, cooled, through a 100 mesh sieve, spare.

4.3

basic alumina. muffle furnace before use after 300 °C calcined 3h, cooled water was added 100g per 5mL, mixing, desiccator over Night and set aside.

4.4

N, O- bis trimethylsilyl trifluoroacetamide.

4.5

trimethylchlorosilane.

4.6

acetonitrile.

4.7

toluene. chromatography.

4.8

Helium. purity $\geq 99.999\%$.

4.9

derivatizing agents. taking N, O- bis trimethylsilyl trifluoroacetamide 99mL, add trimethylsilyl chloride 1mL, mix.

4.10

Alumina column. alumina column chromatography using a plug of glass with a 30mm x 15mm, with the sand core plate G3, charged with an appropriate amount of B to Nitriles, then loaded over anhydrous sodium 1cm high, intermediate high mounted 4cm basic alumina, and then loaded to the top 1cm high sulfate, and gently knock Uniform solid fill, the standby.

4.11 100 μ g/mL clopidol standard stock solution. Weigh accurately clopidol reference substance 10mg, in 100mL flask with methanol solution Solution and dilute to volume, formulated at a concentration of 100 μ g/mL of stock standard solution clopidol. -20 °C below, valid for six months.

4.12 1µg/mL clopidol standard working solution. precise amount of 100µg/mL standard stock solution clopidol 1.0mL, in 100mL Volumetric flask, dilute to the mark with methanol, formulated at a concentration of 1µg/mL of the working standard solution clopidol stored 2 °C ~ 8 °C, valid 1 week.

5 Instruments and equipment

5.1

Gas chromatography - mass spectrometry. with electron impact ionization (EI).

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

high-speed refrigerated centrifuge.

5.5

rotary evaporator.

5.6

shaker.

5.7

polypropylene centrifuge tube. 50mL.

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 Preparation of matrix-matched standard curve

The precise amount of 1µg/mL clopidol appropriate amount of working standard solution, were added to the extracted, the purification step of the processing parts of the blank sample 6 Eluate by derivatization, to obtain the concentration of 5,10,20,100,250 and 500µg/L of matrix-matched series of standard solution, gas phase color Spectroscopy - mass spectrometry. To measure peak area for the vertical axis, corresponding to the concentration of standard solution as abscissa, the standard curve. Seeking regression equation and the correlation coefficient.

7.2 Extract

Sample Weigh 5g ± 0.05g, in 50mL polypropylene centrifuge tube, acetonitrile was added 10mL, homogeneous 1min, oscillation 20min. 4 °C, 6000r/min centrifugal 10min, the supernatant, the residue was extracted with acetonitrile is repeated once 10mL. The combined supernatant twice, adding isopropanol 5mL, 50 deg.] C at a rotary evaporator to near dryness. The residue was dissolved with acetonitrile standby 5mL.

7.3 Purification

Basic alumina column activated with 15mL acetonitrile, taking stock solution through the column, the natural flow of dry acetonitrile was added 10mL, collecting eluate, 50 deg.] C and blown dry with nitrogen.

Derived 7.4

The residue was dissolved in toluene was added 100 L, 100 L adding derivatizing agent, seal, 80 °C derivatization 1h. Cooling, toluene was added 800 L, for Gas Chromatography - Mass Spectrometry.

7.5.1 Gas chromatographic conditions

7.5.1.1

Column. phenylmethyl silicone capillary column (30m x 0.25mm, film thickness 0.25µm), or equivalent person.

7.5.1.2

flow. 0.8mL/min.

7.5.1.3

temperature program. 100 °C 1min, to a rate of 30 °C/min heating to 200 °C, then to 5 °C/min was heated to 205 °C, Paul Temperature 1min, then 30 °C/min was heated to 280 °C, maintained 1min.