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

GB 29686-2013

**Determination of Avilamycin residues in edible tissues of swine
by Liquid Chromatography-tandem Mass Spectrometric method**

食品安全国家标准 猪可食性组织中阿维拉霉素残留量的测定 液相色谱-串联质谱
法

(English Translation)

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1 Scope

This standard specifies the edible tissues of porcine avilamycin Residues sample preparation and liquid chromatography - tandem mass spectrometry.

This standard applies to porcine muscle, fat/skin, liver and kidney detecting residual amounts of avilamycin.

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 Avilamycin remaining in a sample, extracted with acetone and hydrolyzed with sodium hydroxide solution, extracted with ethyl acetate, alumina SPE Purification to dicamba as internal standard, liquid chromatography - tandem mass spectrometry APCI measured internal 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

avilamycin, residue standard marker DIA. content $\geq 98\%$.

4.2

Internal standard. dicamba standard. content $\geq 98\%$.

4.3

Methanol. HPLC grade.

4.4

n-hexane.

4.5

Acetonitrile. chromatographically pure.

4.6

ethyl acetate.

4.7

acetone.

4.8

formic acid.

4.9

sodium hydroxide.

4.10

phosphate.

4.11

SPE column of neutral alumina. 1000mg/6mL, or equivalent person.

4.12 1mol/L sodium hydroxide solution. take sodium hydroxide 4g, dissolve and dilute to 100mL with water.

4.13

Eluent. Take acid 5mL, dissolved in acetonitrile and diluted to 100mL.

4.14 1mg/mL and avilamycin marker residue DIA standard stock solution. accurately

weighed and Avila avilamycin DIA each marker neomycin residue 10mg, respectively 10mL volumetric flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 1mg/mL the residue avilamycin and avilamycin marker DIA standard stock solution. 2 °C ~ 4 °C storage period of one month.

4.15 10µg/mL and the avilamycin marker residue DIA standard working solution. precise amount of 1mg/mL mycophenolic Avila Hormone and residual avilamycin was marked DIA 1.0mL each standard stock solution, respectively, 100mL flask, dissolved in methanol and diluted to Scale, formulated at a concentration of 10µg/mL and the avilamycin marker residue DIA working standard solution. 2 °C ~ 4 °C , Valid for one month.

4.16 1mg/mL internal standard dicamba standard stock solution. Weigh accurately dicamba 10mg, in 10mL flask, Dissolved in methanol and diluted to volume, formulated at a concentration of 1mg/mL of dicamba stock standard solution. 2 °C ~ 4 °C stored, effective 1 month.

4.17 2µg/mL internal standard dicamba standard working solution. precise amount of 1mg/mL dicamba stock standard solution

200 L, in 100mL flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 2µg/mL in the standard work dicamba as a liquid. 2 °C ~ 4 °C storage period of one month.

5 Apparatus

5.1

High Performance Liquid Chromatography - Tandem Mass spectrometer. with atmospheric pressure chemical ionization source (APCI).

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

oscillator.

5.6

vortex mixer.

5.7

homogenizer.

5.8

Nitrogen blowing instrument.

5.9

rotary evaporator.

5.10

SPE.

5.11

round-bottomed flask. 50mL.

5.12

centrifuge tube. 15mL, 50mL.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or frozen 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

Muscle or fat was weighed sample $2g \pm 0.05g$, liver or kidney sample $1g \pm 0.05g$, in 15mL centrifuge tube, and acetone 4 mL, vortexed 5min, at 6000r/min centrifugal 10min, supernatant at 50mL round bottom flask. The residue was added acetone 4mL, the extraction was repeated twice, and the combined supernatant three times, at 60 deg.] C rotary evaporated to dryness, the residue was dissolved with 1mol/L sodium hydroxide solution, 4 mL, Go 50mL centrifuge tube, at 70 deg.] C water bath for 2h, extracted with 85% phosphoric acid adjusted to pH 1.0, was added 4 mL of ethyl acetate, were mixed and 6000r/min centrifugal 10min, supernatant to another centrifuge tube, was repeated twice,