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

GB 29697-2013

**Determination of Diazepam and Methaqualone residues in
animal derived food by Gas Chromatography-Mass
Spectrometric method**

食品安全国家标准 动物性食品中地西洋和安眠酮多残留的测定 气相色谱-质谱法
(English Translation)

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1 Scope

This standard specifies the sample preparation and gas chromatography of animal foods and diazepam methaqualone of Residues - mass spectrometry.

This standard applies to pigs diazepam and methaqualone residues detected in muscle tissue.

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 specimens remaining diazepam and methaqualone, extracted with acetonitrile, hexane degreasing, C18 column purification, gas chromatography - mass spectrometry, external standard method Quantitative.

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

diazepam, methaqualone reference. content $\geq 99.5\%$.

4.2

Acetonitrile. chromatographically pure.

4.3

hexane. chromatography.

4.4

Methanol. HPLC grade.

4.5

toluene. chromatography.

4.6

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

4.7 50% aqueous methanol. Methanol 50mL, dissolved and diluted to 100mL with water.

4.8 70% aqueous methanol. Methanol 70mL, dissolved and diluted to 100mL with water.

4.9 200 μ g/mL methaqualone and diazepam standard stock solution. Precision Weigh methaqualone and diazepam each standard 10mg, respectively, 50mL volumetric flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 200 μ g/mL methaqualone and diazepam standard stock solution.

-20 °C below, valid for six months.

4.10 50 μ g/mL standard stock solution mixed. precise amount of 200 μ g/mL standard stock solution diazepam and 200 μ g/mL standard methaqualone each stock solution 25mL, in 100mL volumetric flask, dilute to volume with methanol, formulated at a concentration of 50 μ g/mL standard stock solution mixed.

-20 °C below, valid for six months.

4.11 10 μ g/mL standard stock solution mixed. precise amount of 50 μ g/mL standard stock solution mixed 10mL, 50mL in the flask, A Alcohol diluted to the mark to prepare a mixed standard stock solution at a concentration of 10 μ g/mL of. -20 °C below, valid for six months.

5 Apparatus

5.1

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

5.2

Analytical balance. a sense of the amount of 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

a vortex.

5.5

centrifuge.

5.6

homogenizer.

5.7

rotary evaporator.

5.8

Nitrogen blowing instrument.

5.9

SPE.

5.10

ultrasonic cleaner.

5.11

heart-shaped bottle. 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 Extract

Sample Weigh $2g \pm 0.02g$, a centrifuge tube, acetonitrile was added 20mL, ultrasonic water bath for 5min. 4000r/min centrifugal 10min, the the supernatant in the heart-shaped flask. At 50 deg.] C and rotary evaporated to near 5mL, 7.5 mL of n-hexane was added, mixing was allowed to stand, the supernatant was discarded. Take the lower liquid at 50 °C nitrogen purge to 1mL, water was added 4mL, mixing spare.

7.2 Purification

C18 column successively with methanol and 3mL 3mL water activation, taking stock solution through the column, washed with 50% aqueous methanol with 3mL, 70% A 3mL aqueous alcohol solution, collecting eluate, blown dry with nitrogen at 50 °C, the residue was dissolved with 200μL of acetonitrile, gas chromatography - mass spectrometry Determination.

7.3 Preparation of standard curve

The precise amount of 10μg/mL working standard solutions amount, diluted with methanol, and formulated to a concentration of 1,10,50,100,500 1000ng/mL standard solution series, gas chromatography - mass spectrometry. Peak area was measured to the vertical axis, the corresponding concentration of the standard solution Degree abscissa, the standard curve. Seeking regression equation and correlation coefficient.

7.4.1 Reference Gas chromatography conditions

7.4.1.1

Gas Chromatographic Column. DB-5MS column (30m x 0.25mm, diameter 0.25μm), or equivalent person.

7.4.1.2

Carrier gas. helium.

7.4.1.3

Carrier gas flow rate. 0.9mL/min.