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

GB 29684-2013

**Determination of Erythromycin residues in aquatic products by
Liquid Chromatography-tandem Mass Spectrometric method**

食品安全国家标准 水产品中红霉素残留量的测定 液相色谱-串联质谱法

(English Translation)

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1 Scope

This standard specifies the aquatic Erythromycin Residues sample preparation and liquid chromatography - tandem mass spectrometry.

This standard applies to edible fish tissues detecting the residual amount of erythromycin.

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 SC/T 3016 aquatic sampling Principle 3 Erythromycin remaining in a sample, extracted with acetonitrile, hexane degreasing, an HLB column purified liquid chromatography - tandem mass spectrometry, internal standard method Quantitative.

4 Reagents and materials

The following reagents used, unless otherwise stated were of analytical reagent; water as a water line with GB/T 6682 provisions.

4.1

erythromycin Standard. Content $\geq 97\%$.

Erythromycin 4.2 isotope internal standard. erythromycin isotope (erythromycinA, N, N-Dimethyl- $^{13}\text{C}_2$) content of $\geq 90\%$.

4.3

Acetonitrile. chromatographically pure.

4.4

hexane. chromatography.

4.5

Methanol. HPLC grade.

4.6

acetic acid.

4.7

sodium chloride.

4.8

twelve disodium hydrogen phosphate water.

4.9

phosphoric acid.

4.10

HLB solid phase extraction column. 200mg/6mL, or equivalent person.

4.11 0.1mol/L phosphate buffer. Take 17.92 g disodium hydrogen phosphate dodecahydrate, dissolve and dilute to 500mL with water, adjusted with phosphoric acid pH to 8.0, now with the current.

4.12 40% methanol solution. Methanol 40mL, dissolved and diluted to 100mL with water.

4.13 30% methanol solution. Methanol 30mL, dissolved and diluted to 100mL with water.

4.14 0.01 per mille acetic acid aqueous solution. Take 10 μL of acetic acid, dissolved and diluted with water to 1000mL.

4.15 100 $\mu\text{g/mL}$ standard stock solution of erythromycin. standard accurately weighed

10mg, in 100mL flask, dissolved in methanol and Diluted to the mark, formulated at a concentration of 100µg/mL standard stock solution of erythromycin. The following -18 °C dark preservation, valid for six months.

4.16 5µg/mL erythromycin standard working solution. precise amount of 100µg/mL standard stock solution of erythromycin 500µL, in 10mL volumetric flask , Diluted with methanol to scale, formulated at a concentration of working standard solution of erythromycin 5µg/mL of. The following -18 °C dark preservation, valid 3 months.

4.17 100µg/mL erythromycin isotope internal standard stock solution. Weigh accurately erythromycin isotope internal standard 10mg, in 100mL flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 100µg/mL erythromycin isotope internal standard stock standard liquid. The following -18 °C dark preservation, valid for six months.

4.18 200ng/mL erythromycin isotope internal standard working standard solution. precise amount of 100µg/mL standard erythromycin isotope internal standard reservoir

200 L was prepared, in 100mL volumetric flask, dilute to the mark with methanol, formulated at a concentration of 200ng/mL in the standard isotope erythromycin Standard working solution. The following -18 °C dark preservation, 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

oscillator.

5.5

homogenizer.

5.6

centrifuge.

5.7

pH meter.

5.8

rotary evaporator.

5.9

eggplant-shaped flask. 100mL.

5.10

centrifuge tube. 50mL.

5.11

filter. 0.22 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Product water test blank or edible 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

-20 °C below Save to 3 months.

Determination Step 7

7.1 Extract

Sample Weigh $5g \pm 0.05g$, in 50mL centrifuge tube, was added 200ng/mL erythromycin isotope internal standard working standard solution 50 L, Acetonitrile was added 15mL, immediately dispersed with stirring, ultrasonic 5min, shaking 5min, 4000r/min centrifugal 10min, supernatant, the other Centrifuge tube, the residue was added acetonitrile 5mL, extraction was repeated once, the two supernatants were combined, and sodium chloride in hexane 2g 15mL, shaking 5min, 4000r/min centrifugal 10min, the upper n-hexane solution was discarded, the lower layer was taken, in 100mL eggplant-shaped flask, to rotary evaporation at 40 °C Dry with phosphate buffer solution 5mL, dissolve the residue, hexane was added 5mL, mixing 1min, transferred to centrifuge tubes, 6000r/min centrifugal 5min, n-hexane layer was discarded, together with n-hexane 5mL, the operation is repeated once, and the lower layer was set aside.