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

GB 29687-2013

**Determination of Albendazole and metabolites residues in
aquatic products by High Performance Liquid Chromatographic
method**

食品安全国家标准 水产品中阿苯达唑及其代谢物多残留的测定 高效液相色谱法
(English Translation)

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1 Scope

This standard specifies albendazole residues and metabolites (2-albendazole sulfone, sulfoxide albendazole, sulfone) in Aquatic the method of sample preparation and determination by HPLC detection.

This standard applies to aquatic Residue albendazole and metabolites (2-albendazole sulfone, sulfoxide albendazole, sulfone) 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 SC/T 3016 aquatic sampling Principle 3 in a sample and the residual metabolite albendazole, extracted with ethyl acetate, hexane degreasing, back-extracted with ethyl acetate, HPLC -Fluorescence detector, 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.

4.1

Preparation of 2-amino-sulfone albendazole, sulfoxide, albendazole sulfone and albendazole reference. content of $\geq 99\%$.

4.2

hexane. chromatography.

4.3

Acetonitrile. chromatographically pure.

4.4

Methanol. HPLC grade.

4.5

ethyl acetate. chromatography.

4.6

dichloromethane. chromatography.

4.7

phosphoric acid.

4.8

twelve disodium hydrogen phosphate water.

4.9

heptane sulfonate. chromatography.

4.10

ammonium acetate.

4.11 20% methanol solution. Methanol 80mL, dissolved and diluted to 100mL with water.

4.12 0.05mol/L ammonium acetate solution. Take ammonium acetate 3.85g, dissolved and diluted with water to 1000mL.

4.13 0.04mol/L of sodium heptane - solution of phosphoric acid. phosphoric acid 2.7 mL take, mixing with water, added 8.08 g sodium heptane, dissolved in water and Diluted to 1000mL.

4.14 3% phosphoric acid solution. Take phosphate 1.75mL, dissolve and dilute to 100mL with water.

4.15 0.02mol/L solution of disodium hydrogen phosphate. disodium-hydrogen phosphate 0.716 g, dissolve and dilute to 100mL with water, 3% phosphoric acid solution PH was adjusted to 8.5, now with the current.

4.16 100µg/mL albendazole, sulfoxide, albendazole sulfone, and 2-amino albendazole sulfone standard stock solution. Weigh accurately A of benzene, albendazole sulfoxide and sulfones of each amino-acid and 10mg 11.5mg albendazole sulfone, respectively in 100mL Flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 100µg/mL standard stock solution. -18 °C below from light, there is Valid for three months.

4.17

working standard solutions. were the precise amount of 100µg/mL 2- amino albendazole sulfone, sulfoxide albendazole, sulfone, and A Standard stock solution of benzene yl amount, in the same flask, dissolve with 80% methanol solution and dilute to volume, formulated at concentrations of 2-amino Albendazole sulfone 1.0µg/mL, albendazole sulfoxide 2.0µg/mL, albendazole sulfone 0.2µg/mL and albendazole 5.0µg/mL of Working standard solutions. 2 °C ~ 8 °C dark storage period of one month.

5 Instruments and equipment

5.1

HPLC. with a fluorescence 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

centrifuge.

5.6

rotary evaporator.

5.7

Nitrogen blowing instrument.

5.8

pear-shaped bottle. 100mL.

5.9

Membrane. organic phase, 0.45µm.

Preparation and Storage of sample 6

6.1 Preparation of the sample

An appropriate amount of fresh or frozen fish, to the scales, peeled, taken along the back muscles; shrimp, to the head, peeled, taking part of the muscle. 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

7.1.1 Extract

Sample Weigh $2g \pm 0.02g$, in 50mL centrifuge tubes, ethyl acetate was added 15mL, homogeneous 30s, shaking 5min, 4000r/min Centrifugal 10min, the supernatant was 100mL pear-shaped flask, the residue was set aside. Another a 50mL centrifuge tubes, ethyl acetate was added 15mL, washed Homogenizer 30s, the residue in the washing liquid, shaken 5min, 4000r/min centrifugal 10min, the supernatant was combined to 100mL pear shaped flask, at 35 °C rotary evaporated to dryness and 1.0mL 20% methanol solution was dissolved the residue.

7.1.2 Degreasing

The above solution was transferred to 10mL centrifuge tube, again pear-shaped flask was added n-hexane 1mL, washed with n-hexane into a centrifuge tube, vibration Swing 1min, 4000r/min centrifugal 5min, n-hexane layer was discarded, and then n-hexane was added 1mL, operation was repeated twice, and the lower layer was reserved.

7.1.3 Purification

Stock solution was added 0.04mol/L phosphoric acid 1mL, mix, add 1.5 mL of dichloromethane, mixed 1min, 4000r/min centrifugal 5min, Methylene chloride was taken in 10mL centrifuge tube, together with 1.5 mL of dichloromethane, extraction was repeated twice, three times with dichloromethane were combined in 10mL Centrifuge tube, 35 °C dry nitrogen; with 0.02mol/L of disodium hydrogen phosphate solution 1.0mL dissolve the residue, ethyl acetate was added 2mL, mixed Together 1min, 4000r/min centrifugal 5min, ethyl acetate was transferred to another 10mL centrifuge tube, followed by adding disodium hydrogen phosphate was added Ethyl acetate 2mL, extraction was repeated twice, ethyl acetate three times were combined, dried nitrogen at 40 °C, with 20% methanol - water