

ICS 67.020

CCS C 53



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

GB 29695-2013

**Determination of Avermectin and Ivermectin residues in aquatic
products by High Performance Liquid Chromatographic method**

食品安全国家标准 水产品中阿维菌素和伊维菌素多残留的测定 高效液相色谱法

(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 Apparatus
6.1 Preparation of the sample
7.1 Preparation of standard curve
7.2 Extract
7.3 Purification
7.4 Derivatization
7.5 Determination
7.6 Blank test
8 Results calculated
9 Method sensitivity, accuracy and precision
9.1 Sensitivity
9.2 Accuracy
9.3 Precision

1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method aquatic avermectin and ivermectin residues.

Abamectin and ivermectin detecting residual amounts of edible fish is applicable to the organization's.

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 avermectin and ivermectin, extracted with acetonitrile, degreasing hexane, basic alumina column purification, N- methylimidazole, and Trifluoroacetic anhydride derivatization, high performance liquid chromatography - fluorescence determination, external standard.

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

avermectin standard. B1a content of $\geq 94\%$. Ivermectin Standard. B1a content of $\geq 92\%$.

4.2

Acetonitrile. chromatographically pure.

4.3

Methanol. HPLC grade.

4.4

n-hexane.

4.5

trifluoroacetic anhydride.

4.6

N- methylimidazole.

4.7

acetic acid. excellent pure.

4.8

over anhydrous sodium. 650 °C drying 4h, cool in a desiccator for use.

4.9

SPE cartridge of basic alumina. 1g/3mL, or equivalent person.

4.10

derivatizing agent A. N- methyl imidazole take 1mL, acetonitrile 1mL, mix, now with the current.

4.11

derivatizing agent B. trifluoroacetic anhydride take 1mL, acetonitrile 2mL, mix, now with the current.

4.12 0.4% acetic acid solution. 0.4mL harvest acid, dissolve and dilute to 100mL with water.

4.13 100 μ g/mL abamectin and ivermectin mixed standard stock solution. Weigh accurately abamectin and ivermectin each standard 10mg, in 100mL flask, dissolved in methanol and diluted to the mark, formulated at a concentration of 100 μ g/mL of Ivermectin and

Abamectin Element mixed standard stock solution. -18 °C below, valid for six months.

4.14 10µg/mL abamectin and ivermectin working standard solutions. precise amount of 100µg/mL abamectin and ivermectin mixed Combined standard stock solution 10mL, in 100mL volumetric flask, dilute to the mark with methanol, formulated at a concentration of 10µg/mL abamectin and Ivermectin working standard solutions. 2 °C ~ 8 °C storage period of three months.

5 Apparatus

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

vortex mixer.

5.5

ultrasonic cleaning.

5.6

centrifuge.

5.7

Nitrogen blowing instrument.

5.8

rotary evaporator.

5.9

SPE.

5.10

stopper glass centrifuge tube. 5mL.

5.11

over anhydrous sodium Column. Glass column (phi10mm x 150mm, No. 2 sand core) was charged with 5g of anhydrous sodium sulfate.

5.12

eggplant-shaped bottle. 50mL.

5.13

stopper polypropylene centrifuge tube. 50mL.

5.14

filter. 0.45 μ m.

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

-18 °C below saved.

Determination Step 7

7.1 Preparation of standard curve

The precise amount of 1 μ g/mL abamectin and ivermectin working standard solutions amount, diluted with methanol to prepare a concentration of 0.1, 0.2, 0.5 and 2 μ g/mL of the series of standard solution from each centrifuge tube 0.1mL to 5mL, blowing at 45 °C ~ 55 °C water bath nitrogen Dryness, treated according to the derivatization step. For HPLC. To measure peak area for the vertical axis, corresponding to the concentration of standard solution of abscissas Standard, the standard curve. Seeking regression equation and correlation coefficient.

7.2 Extract

Sample Weigh 5g $\pm$ 0.05g, in 50mL polypropylene centrifuge tube, acetonitrile was added 15mL, vortexed 1min, ultrasonic 30min, 4000r/min centrifugal 5min, supernatant to another 50mL polypropylene centrifuge tube. The residue was added acetonitrile 10mL, extraction was repeated Once, the combined two extracts. In the extract was added hexane 10mL, vortexed thoroughly mixed 1min, 4000r/min centrifugal 5min, discarded the upper hexane layer. The combined hexane extracts 10mL, extraction was repeated once, hexane layer was discarded, the standby.