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A 21

GB/T 38095-2019

**Determination of docosahexaenoic acid and eicosapentaenoic
acid content - Gas chromatography**

DHA、EPA 含量测定 气相色谱法

Issued on: October 18, 2019

Implemented on: October 18, 2019

**Issued by: State Administration for Market Regulation;
Standardization Administration of PRC.**

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Determination of docosahexaenoic acid and eicosapentaenoic acid content - Gas chromatography

1 Scope

This standard specifies the method for the determination of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content by gas chromatography.

This standard is applicable to the determination of DHA and EPA content in fish oil and microalgae.

This standard does not apply to the determination of ethyl-esterified fish oil.

The detection limit of this method is 10 mg/kg, and the quantification limit is 33 mg/kg.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use. Specification and test methods

3 Principles

After extracting oil from the sample by chloroform-methanol method, methyl-esterify the sample and separate it with a gas chromatographic capillary column; then, inspect it with a hydrogen flame ionization detector and quantify it by the internal standard method.

4 Reagents or materials

Unless otherwise specified, only analytical reagents are used.

4.1 Water: Grade 1 specified in GB/T 6682.

4.2 Methanol: chromatographically pure.

4.3 n-hexane: chromatographically pure.

Microalgae: Weigh 10.00 g of microalgae and grind, then weigh 1.00 g of uniform sample, which will be used for the extraction of oil.

Fish oil: take 0.1000 g.

6.2 Extraction

Take 1.00 g of microalgae dry powder sample, and put it into a 50 mL polytetrafluoroethylene test tube; add 6.0 mL of distilled water, add 22.5 mL of chloroform-methanol (1:2, volume ratio) mixed solution, shake and mix well; then, disrupt it with an ultrasonic cell disruptor for 2 min, add 7.5 mL of chloroform, shake and mix for 30 s; add 7.5 mL of distilled water, shake and mix for 30 s. Transfer the mixture to a separatory funnel, let it be standing and layering, and separate the oil layer; then, dry it in a vacuum, weigh it and calculate the oil content.

6.3 Fatty acid methyl esterification

Weigh 0.10 g of the oil sample, and put it into a 10 mL centrifuge tube with a lid; add 2 mL of ether-n-hexane (2:1, volume ratio) mixture, and shake well; 15 min later, add 2 mL of 0.8 mol/L potassium hydroxide-methanol solution, shake well, and let it react in a constant temperature water bath at 60 °C for 15 min; then, add water along the wall to the 10 mL mark, and let it be standing and layering; transfer all the supernatant to a measuring apparatus, dilute to 1 mL with n-hexane; dilute it by a certain multiple, and let it be injected and analyzed later.

6.4 Determination

6.4.1 Gas chromatography reference conditions

The gas chromatography reference conditions are as follows:

- a) Chromatographic column: poly(dicyanopropyl siloxane) capillary column (0.32 mm×0.25 μm×30 m), or equivalent;
- b) Carrier gas and flow rate: The carrier gas shall be nitrogen, and the flow rate shall be 1.5 mL/min;
- c) Column temperature raising procedure: The initial temperature shall be 160 °C (hold for 3 min), then rise to 230 °C at a rate of 6 °C/min and hold for 15 min;
- d) Injection port temperature: 250 °C;
- e) Detector temperature: 280 °C;
- f) Injection volume: 1 μL;
- g) Injection method: split injection; split ratio shall be 50:1.

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