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

GB 28404-2012

**National food safety standards in health food γ -linolenic acid,
eicosapentaenoic acid, docosapentaenoic acid and 22 carbon
60-acid determination**

保健食品中 α -亚麻酸、二十碳五烯酸、二十二碳五烯酸和二十二碳六烯酸的测定
(English Translation)

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1 Scope

This standard specifies the health food alpha-linolenic acid, eicosapentaenoic acid (referred to as EPA, the same below), docosahexaenoic acid (referred to as DPA, the same below) and docosahexaenoic acid (hereinafter referred to as DHA, the same below) by gas chromatography.

This standard is applicable to the determination of alpha-linolenic acid, EPA, DPA and DHA in health food products, and is not suitable for the determination of fatty acid Determination of alpha-linolenic acid, EPA, DPA, DHA in health food.

2 Principle

The samples were extracted by acid hydrolysis to extract fats, including alpha-linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DPA), twenty-two Carboxyhexaenoic acid (DHA) was transesterified to form methyl ester, which was separated by gas chromatography to determine the time qualitative and external standard method.

3 Reagents and materials

Note: Unless otherwise stated, the reagents used in this method are of analytical grade and water is a primary water specified in GB/T 6682.

3.1 Reagents

3.1.1

Potassium hydroxide (KOH).

3.1.2

hydrochloric acid (HCl).

3.1.3

anhydrous ether (C₂H₅OC₂H₅).

3.1.4

Ethanol (CH₃CH₂OH). volume fraction $\geq 95\%$.

3.1.5

petroleum ether. boiling range 30 °C ~ 60 °C.

3.1.6

n-Hexane [CH₃ (CH₂)₄CH₃]. Chromatographic Purification.

3.1.7

Methanol (CH₃OH). Chromatographic Purification.

3.1.8

anhydrous sodium sulfate (Na₂SO₄).

3.2 Reagent preparation

Potassium hydroxide solution of methanol (0.5mol/L). Weigh 2.8g potassium hydroxide, dissolved in methanol and volume to 100mL, and mix.

3.3 Standards

3.3.1

alpha-linolenic acid methyl ester (C₁₉H₃₂O₂). purity $\geq 99.0\%$.

3.3.2

EPA methyl ester (C₂₁H₃₂O₂). purity $\geq 98.5\%$.

3.3.3

DPA methyl ester (C₂₃H₃₆O₂). purity $\geq$ 98.0%.

3.3.4

DHA methyl ester (C₂₃H₃₄O₂). purity $\geq$ 98.5%.

3.4 Preparation of standard solution

3.4.1

Single Fatty Acid Methyl Ester Standard Stock Solution (4.0 mg/mL). Weigh 100.0 mg alpha-linolenic acid methyl ester, EPA methyl ester, DPA A Ester, DHA methyl ester standard material in 25.0mL volumetric flask, respectively, with n-hexane dissolved and fixed to the scale, shake. This solution should be stored in -18 °C refrigerator.

3.4.2

Fatty Acid Methyl Ester Mix Standard Intermediate (1.0mg/mL). Separate Fatty Acid Methyl Ester Standard Stock 2.50mL in 10.0mL Capacity bottle, shake, but also for the highest standard curve of the standard, when the preparation of temporary use.

3.4.3

fatty acid methyl ester standard working solution. respectively, to absorb fatty acid methyl ester intermediate 0.40mL, 0.80mL, 1.0mL, 2.0mL, 4.0 mL in a 10 mL volumetric flask, with a volume of 0.040 mg/mL, 0.080 mg/mL, 0.10 mg/mL, 0.20mg/mL, 0.40mg/mL standard working solution, when used in preparation.

4 Instruments and equipment

4.1

Gas Chromatograph. with a hydrogen flame ionization detector (FID).

4.2

balance. sense of 1mg and 0.1mg.

4.3

Rotary Evaporator.

4.4

Centrifuge. speed $\geq$ 4000r/min.

4.5

Scroll Mixer.

4.6

constant temperature water bath.

5.1.1.1 Solid samples

Weigh the pulverized and mixed samples to be tested 0.5g ~ 2g (accurate to 0.001g) (including the measured components 5mg/g ~ 10mg/g) plus Into the 50mL colorimetric tube, add 8mL water, mix and add 10mL hydrochloric acid. The color tube into the 70 °C ~ 80 °C water bath, every time 5min ~ 10min with a vortex mixer mixed once, until the sample hydrolysis is complete, about 40min ~ 50min. Remove the color tube, add Into 10mL of ethanol, mixed. After cooling to room temperature, the mixture was transferred to a 100 mL stopper cylinder, washed with 25 mL of anhydrous ether Color tube, into the cylinder together. Dense plug shaking 1min. Add 25mL petroleum ether, dense plug shaking 1min, standing 30min, stratified, will the extracted organic layer was passed through an anhydrous sodium sulfate (about 5 g) into a concentration flask. Then add 25mL anhydrous ether dense plug shaking 1min, 25mL Petroleum ether, dense plug shaking 1min, standing, stratified, the aspirated organic layer through anhydrous sodium sulfate (about 5g) into the concentration of the bottle. Press "plus Into 25 mL of anhydrous ether, let stand, layer, the organic layer of the suction through anhydrous sodium sulfate (about 5g) filter into the bottle "repeat operation at one time, the whole extract was concentrated to 45 ° C under a rotary evaporator at reduced pressure to near dryness. Dissolve the concentrate a few times with n-hexane and transfer to 25mL volumetric flask and volume, shake. Methylation in accordance with 5.1.2.

5.1.1.2 Oil products

Weigh the mixed oil products 0.2g ~ 1g (accurate to 0.001g) (including the measured components 10mg/g ~ 20mg/g) to 25mL Capacity flask, add 5mL n-hexane shake to dissolve, and n-hexane volume to the mark, shake. According to 5.1.2 step methyl esterification treatment, fat for the physical identification of ethyl ethyl ester type oils, see Appendix A.

5.1.2 Methylation

Absorb the test solution (5.1.1.1, 5.1.1.1) 2.0mL to 10mL with a plug scale tube, add 2.0mL potassium hydroxide methanol solution Liquid, immediately moved to the vortex mixer for 5min oscillation, put it aside for 5min, add 6mL distilled water, shaking up and down for 0.5min, Layer, the lower layer of liquid, discard and then repeated with a small amount of distilled water for washing, and with a straw to abandon the water layer until the wash to neutral (if organic Phase with the phenomenon of emulsification to 4000r/min centrifugal 10min), the absorption of n-hexane to be on the machine test.

Note: If the use of plastic centrifuge tube or plastic scale tube for sample processing should be