

中华人民共和国出入境检验检疫行业标准

SN/T 2922—2022
代替 SN/T 2922—2011

出口保健食品中 EPA、DHA 和 AA 的测定 气相色谱法

Determination of eicosapentaenoic acid (EPA)、docosahexaenoic acid (DHA) and
Arachidonic acid(AA)in health foods for export—Gas chromatography

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前　　言

本文件按照 GB/T 1.1—2020《标准化工作导则 第1部分：标准化文件的结构和起草规则》的规定起草。

本文件代替 SN/T 2922—2011《出口食品中 EPA 和 DHA 的测定 气相色谱法》，与 SN/T 2922—2011 相比，主要技术变化如下：

——更改了标准名称；

——检测对象增加了顺式-5,8,11,14-二十碳四烯酸(AA)的测定。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别专利的责任。

本文件由中华人民共和国海关总署提出并归口。

本文件起草单位：中华人民共和国广州海关。

本文件主要起草人：陈捷、叶弘毅、孙灵慧、徐娟、王岚。

本文件及其所代替文件的历次版本发布情况为：

——2011 年首次发布为 SN/T 2922—2011；

——本次为第一次修订。

出口保健食品中 EPA、DHA 和 AA 的测定 气相色谱法

1 范围

本文件规定了出口保健食品中顺式-5,8,11,14,17-二十碳五烯酸(简称 EPA)、顺式-4,7,10,13,16,19-二十二碳六烯酸(简称 DHA)和顺式-5,8,11,14-二十碳四烯酸(简称 AA)的气相色谱测定方法。

本文件适用于出口鱼油和鱼油复合胶囊制品中 EPA、DHA、AA 含量的测定。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中,注日期的引用文件,仅该日期对应的版本适用于本文件,不注日期的引用文件,其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682 分析实验用水规格和试验方法。

3 术语和定义

本文件没有需要界定的术语和定义。

4 方法提要

样品中 EPA 甘油酯、DHA 甘油酯、AA 甘油酯和游离的 EPA、DHA、AA 经氢氧化钠甲醇溶液和三氟化硼甲醇溶液甲酯化,异辛烷提取,采用气相色谱配氢火焰离子检测器(FID)检测,内标法定量。

5 试剂和材料

除另有规定外,所有试剂均为分析纯,所用试剂对测定无干扰,实验用水为符合 GB/T 6682 规定的一级水。

5.1 异辛烷。

5.2 甲醇。

5.3 12%三氟化硼甲醇溶液。

5.4 氢氧化钠。

5.5 氯化钠。

5.6 氢氧化钠甲醇溶液(0.5 mol/L):称取 2.0 g 氢氧化钠,加 100 mL 甲醇溶解。

5.7 饱和氯化钠溶液:36 g 氯化钠,溶于 100 mL 水中。

5.8 标准物质

5.8.1 二十三酸甲酯: CAS No. 2433-97-8, 相对分子质量为 368.64, 纯度 \geqslant 99.0%, 内标物。

5.8.2 EPA 甲酯: CAS No. 2734-47-6, 相对分子质量为 316.48, 纯度 \geqslant 99.0%。

5.8.3 DHA 甲酯: CAS No. 2566-90-7, 相对分子质量为 342.51, 纯度 \geqslant 99.0%。

5.8.4 AA 甲酯: CAS No. 2566-89-4, 相对分子质量为 318.49, 纯度 $\geqslant 99.0\%$ 。

5.9 标准溶液配制

5.9.1 内标二十三酸甲酯储备液: 准确称取适量二十三酸甲酯(精确到 0.000 1 g), 用异辛烷溶解, 配制成浓度为 1.00 mg/mL 的内标储备液。

5.9.2 EPA 甲酯储备液: 准确称取适量 EPA 甲酯(精确到 0.000 1 g), 用异辛烷配制成浓度为 100.0 mg/mL 的标准储备液。

5.9.3 DHA 甲酯储备液: 准确称取适量 DHA 甲酯(精确到 0.000 1 g), 用异辛烷配制成浓度为 100.0 mg/mL 的标准储备液。

5.9.4 AA 甲酯储备液: 准确称取适量 AA 甲酯(精确到 0.000 1 g), 用异辛烷配制成浓度为 100.0 mg/mL 的标准储备液。

注: 上述标准溶液应于-18 ℃冰箱中保存。

6 仪器和设备

6.1 气相色谱仪: 配有氢火焰离子检测器(FID)。

6.2 天平: 感量 0.000 1 g。

6.3 旋涡震荡器。

6.4 烘箱: 25 ℃~200 ℃, ± 1 ℃。

6.5 氮气吹干仪。

6.6 甲酯化反应瓶: 带聚四氟乙烯垫旋盖的顶空进样瓶或玻璃试管, 25 mL。

6.7 玻璃刻度试管: 5.0 mL, 具塞。

7 测定步骤

7.1 准确移取内标二十三酸甲酯储备液(5.9.1) 2.0 mL(相当于内标物 2.0 mg)于甲酯化反应瓶(6.6)中, 用氮气吹干, 待用。如果不马上使用, 甲酯化反应瓶应于-18 ℃冷冻储存。

7.2 称取试样(胶囊样品取内含物)0.02 g(精确至 0.000 1 g)于含有内标二十三酸甲酯储备液的甲酯化反应瓶中(7.1), 加 1.5 mL 0.5 mol/L 氢氧化钠甲醇溶液(5.6), 充入氮气, 加盖密封, 旋涡震荡混匀, 置于 100 ℃烘箱内, 加热 5 min, 冷却, 加 2 mL 12% 三氟化硼甲醇溶液(5.3), 充入氮气, 加盖密封, 旋涡震荡混匀, 置于 100 ℃烘箱内, 加热 30 min, 冷却至 30 ℃~40 ℃, 注入 1 mL 异辛烷, 趁热涡旋振荡 30 s, 再注入 5 mL 饱和氯化钠溶液(5.7), 涡旋振荡, 冷却至室温, 静置分层, 将异辛烷层移入玻璃刻度试管, 充入氮气, 加盖。在水相中再加入 1 mL 异辛烷, 重复萃取一次, 合并异辛烷提取液, 通氮气浓缩至 1.0 mL, 待测。

7.3 标准系列工作液配制: 准确移取 EPA 甲酯(5.9.2)、DHA 甲酯(5.9.3)和 AA 甲酯(5.9.4)适量, 再在每个容量瓶中加入相同量的内标适量, 用异辛烷定容, 配制成标准系列工作液, EPA 甲酯、DHA 甲酯和 AA 甲酯的浓度依次为 0.02 mg/mL、0.5 mg/mL、1.0 mg/mL、2.0 mg/mL、4.0 mg/mL、10.0 mg/mL, 内标浓度均为 2.0 mg/mL。标准工作曲线溶液现用现配。

7.4 测定

7.4.1 气相色谱条件

7.4.1.1 色谱柱: DB-23 毛细管柱, 60 m×0.25 mm(i. d.)×0.15 μm , 或性能相当者。

7.4.1.2 升温程序: 150 ℃(2 min) $\xrightarrow{3^\circ\text{C}/\text{min}}$ 200 ℃ $\xrightarrow{2^\circ\text{C}/\text{min}}$ 220 ℃(2 min) $\xrightarrow{25^\circ\text{C}/\text{min}}$ 230 ℃(2 min)。

7.4.1.3 进样口温度: 250 ℃。

7.4.1.4 检测器温度: 280 ℃。

7.4.1.5 载气:氮气(纯度99.999%),流量2.0 mL/min。

7.4.1.6 进样模式:分流进样,分流比 30 : 1。

7.4.1.7 进样量:1.0 μ L。

7.4.2 气相色谱测定

内标法定量：标准工作溶液(7.3)和样液等体积参插进样测定。以标准溶液中被测组分峰面积和二十三酸甲酯峰面积的比值为纵坐标，标准溶液中被测组分浓度与二十三酸甲酯浓度的比值为横坐标绘制标准工作曲线，用标准工作曲线对样品进行定量，标准工作溶液和样液中待测物响应值均应在仪器检测线性范围内。在上述色谱条件下，AA 甲酯的保留时间约为 20.2 min，EPA 甲酯的保留时间约为 21.3 min，DHA 甲酯的保留时间约为 25.4 min，内标二十三酸甲酯的保留时间约为 22.9 min。标准品的色谱图参见附录 A 中图 A.1。

8 结果计算和表述

用气相色谱数据处理软件或按照式(1)计算试样中 EPA 或 DHA 或 AA 的含量:

$$X = \frac{C_s \times A \times c_i \times A_{si} V \times k}{A_s \times c_{si} \times A_i \times m} \quad \dots \dots \dots \quad (1)$$

式中：

X ——试样中 EPA 或 DHA 或 AA 含量, 单位为毫克每克(mg/g);

c_s —— 标准溶液中 EPA 甲酯或 DHA 甲酯或 AA 甲酯的浓度, 单位为毫克每毫升(mg/mL);

A ——试样中 EPA 甲酯或 DHA 甲酯或 AA 甲酯的峰面积；

c_i ——试样溶液中内标二十三酸甲酯的浓度,单位为毫克每毫升(mg/mL);

A_{si} ——标准溶液中内标二十三酸甲酯的峰面积；

V ——最终样液的定容体积,单位为毫升(mL);

k ——0.956 或 0.959;0.956 为 EPA 甲酯和 AA 甲酯换算为 EPA 和 AA 的换算因子;0.959 为 DHA 甲酯换算为 DHA 的换算因子;

A_s ——标准溶液中 EPA 甲酯或 DHA 甲酯或 AA 甲酯的峰面积；

c_{si} ——标准溶液中内标二十三酸甲酯的浓度,单位为毫克每毫升(mg/mL);

A_i ——试样中内标三十三酸甲酯的峰面积；

m ——最终样液所代表试样量,单位为克(g)。

注：计算结果应表示到小数点后一位。

9 定量限和回收率

9.1 定量限

本方法 EPA、DHA 和 AA 的定量限均为 1.0 mg/g。

9.2 回收率

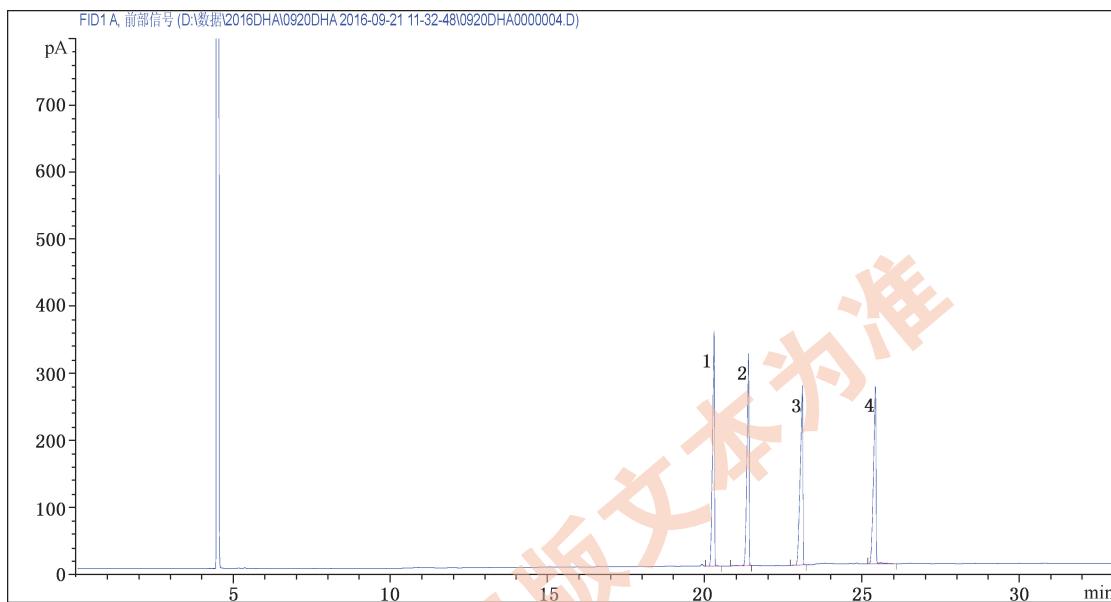
在各种鱼油中,EPA、DHA 和 AA 的回收率范围分别为 97.1%~102%、97.2%~102% 和 97.0%~103%, 参见附录 B。

附录 A

(资料性)

EPA 甲酯、DHA 甲酯和 AA 甲酯的标准品气相色谱图

EPA 甲酯、DHA 甲酯和 AA 甲酯的标准品气相色谱图如图 A.1 所示。



标引序号说明：

- 1——AA 甲酯；
- 2——EPA 甲酯；
- 3——内标二十三酸甲酯；
- 4——DHA 甲酯。

图 A.1 EPA 甲酯、DHA 甲酯和 AA 甲酯的标准品气相色谱图(GC-FID)

附录 B
(资料性)
EPA、DHA 和 AA 的回收率数据

EPA、DHA 和 AA 的回收率数据见表 B. 1。

表 B. 1 EPA、DHA 和 AA 的回收率

样品名称	添加水平 mg/g	回收率范围/%		添加水平 mg/g	回收率范围/%
		EPA	DHA		
深海鱼油胶囊	50.0	97.4~100	97.6~101	10.0	97.6~102
	100	98.1~99.6	97.8~101	50.0	97.5~102
	200	98.8~100	98.7~101	100	98.4~101
鱼油 DHA 提取物	50.0	98.0~101	97.5~101	10.0	98.2~102
	100	99.2~101	98.0~100	50.0	98.0~102
	200	99.1~101	99.6~101	100	99.0~101
鱼油 EPA 提取物	50.0	97.1~102	98.0~102	10.0	97.4~103
	100	98.8~101	98.2~101	50.0	97.7~101
	200	99.1~101	98.8~101	100	98.1~101
金枪鱼鱼油	50.0	98.3~101	97.6~101	10.0	97.4~102
	100	98.7~101	98.3~101	50.0	99.0~102
	200	99.1~101	98.5~100	100	99.8~102
三文鱼鱼油	50.0	98.2~102	97.2~102	10.0	97.0~102
	100	98.5~101	98.5~101	50.0	98.0~101
	200	98.8~101	98.8~101	100	98.2~101
海藻 DHA 提取物	50.0	98.0~102	98.0~103	10.0	97.5~101
	100	98.2~101	98.9~101	50.0	98.0~102
	200	99.1~101	99.3~101	100	98.2~100
橄榄油	10	97.6~101	97.8~104	10	97.1~102
	50.0	97.3~102	97.8~103	50.0	99.8~103
	100	97.6~101	98.5~102	100	98.7~102
	200	98.8~101	98.6~101	200	98.7~101

Foreword

This document is in accordance with GB/T 1.1—2020.

This document supersedes the SN/T 2922—2011 *Determination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in foods—Gas chromatography*.

The main improvement from SN/T 2922—2011:

- The name of the standard is changed.
- Revised content is increased by the determination of arachidonic acid (AA)¹⁾.

Please note that certain elements of this document may relate to patents, and the issuer of this document is not responsible for identifying such patents.

This document is put forward and promulgated by the General Administration of Customs, P. R. C.

The drafting unit of this document: Guangzhou Customs of the People's Republic of China.

The main drafters of this document are Chen Jie, Ye Hongyi, Sun Linghui, Xu Juan, Wang Lan.

This document and the previous releases of its replacement documents are as follows:

- It was first released as SN/T 2922—2011 in 2011;
- This document is the first revision.

Determination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and Arachidonic acid (AA) in health foods for export—Gas chromatography

1 Scope

This document specifies the determination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and Arachidonic acid (AA) in encapsulated fish oil and its composite products for import by gas chromatography.

This document is applicable to the determination of EPA and DHA and AA in encapsulated fish oil and its composite products for export.

2 Normative reference

The following normative documents contain provisions which, through reference in this text, constitute provisions of this document. For dated references, subsequent amendments to, or revisions of any of these publications do not apply. However parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

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

3 Terms and definitions

Terms and definitions are not defined in the document.

4 Principle

Test portions are weighed into Teflon-lined screw-cap glass tubes that contain appropriate internal standard ($C_{23,0}$ methyl ester). Fatty acids include EPA and DHA and AA of oils are derivatized to methyl esters by reacting with methanolic NaOH and BF₃ in methanol. EPA and DHA are determined by gas chromatograph with flame ionization detector and quantitatively determined using internal

standard method.

5 Reagents and materials

Unless otherwise specified, all reagents used should be analytical grade. The reagents used did not interfere with the determination . “water” is complied with GB/T 6682 provides.

5.1 Isooctane.

5.2 Methanol.

5.3 Boron trifluoride. , 12% in methanol.

5.4 Sodium hydroxide.

5.5 Sodium chloride.

5.6 Alcoholic sodium hydroxide.. —0. 5 mol/L. Dissolve 2. 0 g NaOH in methanol and dilute to 100 mL with methanol.

5.7 Sodium chloride. —Saturated solution. Dissolve 36 g NaCl in 100 mL water.

5.8 Reference materials

5.8.1 C_{23:0} methyl ester. — CAS No. 2433-97-8. Reagent of 99 + % purity.

5.8.2 4. 8. 2 all-cis-5,8,11,14,17-eicosapentaenoic acid methyl ester — CAS No. 2734-47-6. Reagent of 99 + % purity.

5.8.3 all-cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester —CAS No. 566-09-7. Reagent of 99 + % purity.

5.8.4 all-cis-5,8,11,14- arachidonic acid methyl ester —CAS No. 2566-89-4. Reagent of 99 + % purity.

5.9 Preparation of Standards

5.9.1 C_{23:0} methyl ester internal standard solution

Accurately weigh adequate amount of C_{23:0} methyl ester standard (accurate to 0. 000 1 g) , dissolve with isooctane and prepare a solution of 1. 00 mg/mL as internal standard solution.

5.9.2 EPA methyl ester standard stock solution

Accurately weigh adequate amount of all-cis-5,8,11,14,17-eicosapentaenoic acid methyl ester standard (accurate to 0.000 1 g), dissolve with isoctane and prepare a solution of 100.00 mg/mL as standard stock solution.

5.9.3 DHA methyl ester standard stock solution

Accurately weigh adequate amount of all-cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester standard (accurate to 0.000 1 g), dissolve with isoctane and prepare a solution of 100.00 mg/mL as standard stock solution.

5.9.4 AA methyl ester standard stock solution

Accurately weigh adequate amount of all-cis-5,8,11,14- arachidonic acid methyl ester standard (accurate to 0.0001 g), dissolve with isoctane and prepare a solution of 100.00 mg/mL as standard stock solution.

NOTE All the standard solutions should be stored at -18°C in a refrigerator.

6. Apparatus and equipment

6.1 Gas chromatograph.—With flame ionization detector, capillary column injection system.

6.2 Analytical balance.—Readability $\pm 0.000 1$ g.

6.3 Vortex mixer.

6.4 Oven. 25°C ~200°C, ± 1 °C.

6.5 Nitrogen evaporator.

6.6 Methyl ester reaction bottle; Head-space injection vials or glass tubes, 25 mL, with leak-tight, Teflon-lined screw caps.

6.7 Glass tubes with scale, 5mL ,with stoppers.

7. Procedures

7.1 Accurately pipet 2.0 mL of C_{23:0} methyl ester (5.9.1) internal standard solution into vials(6.6)

and evaporate solvent in gentle stream of N₂. If not used immediately, it should be refrigerated at -18 °C.

7.2 Accurately weigh ca 0.020 g (± 0.0001 mg) oil into a vial containing methyl ester IS(7.1). Add 1.5 mL 0.5 mol/L methanolic NaOH(5.6), blanket with nitrogen, cap, mix on vortex mixer, and heat 5 min at 100 °C. Cool, add 2 mL BF3 in methanol(5.3), blanket with N₂, cap tightly, mix, and heat 30 min at 100 °C. Cool mixture to 30°~40 °C, add 1 mL isoctane, blanket with N₂, cap, and shake vigorously for 30 s while still warm. Immediately add 5 mL saturated NaCl solution(5.7), blanket with N₂, cap, and agitate thoroughly. Cool to room temperature. When isoctane layer separates from aqueous lower phase, transfer isoctane layer to a clean glass tube, blanket with N₂, and cap. Extract aqueous lower phase a second time with an additional 1 mL isoctane. Combine isoctane extracts and concentrate to ca 1 mL in stream of dry N₂.

7.3 Preparation of standard working solutions

Accurately pipe a serials amount of EPA methyl ester and DHA methyl ester and AA methyl ester standard stock solutions(5.9.2, 5.9.3, 5.9.4) into vials containing IS (5.1), the amount of EPA methyl ester and DHA methyl ester and AA methyl ester are 0.02、0.5、1.0、2.0、4.0、10 mg , respectively. Then diluted with isoctane to make standard working solution mixtures of EPA methyl ester and DHA methyl ester and AA methyl ester, which concentration are 0.02、0.5、1.0、2.0、4.0、10.0 mg/mL, respectively. The concentration of IS is 2 mg/mL. Prepare it before use.

7.4 Determination

7.4.1 GC operating conditions

7.4.1.1 GC column: Capillary column, DB-23, 60 m × 0.25 mm(i. d.) × 0.25 m or equivalent column.

7.4.1.2 Oven temperature programme:

150°C (hold 0.5 min) $\xrightarrow{30\text{ }^{\circ}\text{C}/\text{min}}$ 200 °C $\xrightarrow{2\text{ }^{\circ}\text{C}/\text{min}}$ 220 °C (hold 2 min) $\xrightarrow{25\text{ }^{\circ}\text{C}/\text{min}}$ 230 °C (hold 2 min).

7.4.1.3 Injection port temperature: 250 °C .

7.4.1.4 Detector temperature: 245 °C .

7.4.1.5 Carrier gas: nitrogen, purity $\geqslant 99.999\%$; Flow rate: 2.0 mL/min.

7.4.1.6 Injection mode: split mode preferred at split ratio of 1 : 30;

7.4.1.7 Injection volume; 1 µL.

7.4.2 GC determination

Internal standard method quantitation: Inject standard working solutions (7.3) and sample solutions alternatively. The calibration curve is made as follows: The area ratio of target peak to the IS is used as y-axis, the concentration ratio of target compound to the IS is used as abscissa. The concentration of target compounds in sample solutions were calculated from the calibration curve. The responses of EPA methyl ester and DHA methyl ester and AA methyl ester in the standard working solution and sample solution should be in the linear range of the instrumental detection. At the above GC conditions, the retention time of AA methyl ester and DHA methyl ester and EPA methyl ester are 20.2 min and 21.3 min and 25.4 min, respectively. The GC chromatogram of AA methyl ester and EPA methyl ester and DHA methyl ester standard see annex A.

8. Calculation and expression of result

The content of EPA and DHA and AA in the test sample are calculated by GC data processor or according to the formula (1):

Where:

X —The content of EPA or DHA or AA in the test sample, mg/g;

c_s —The concentration of EPA or DHA or AA (methyl ester) in standard solution, mg/mL;

A —The peak area of EPA or DHA or AA (methyl ester) in the test sample;

c_i —The concentration of C_{23:0} methyl ester internal standard in the test sample, mg/mL;

A_{si} —The peak area of C_{23:0} methyl ester internal standard in standard solution;

V —The final volume of the sample solution, mL;

k —Factor necessary to express result as mg fatty acid/g oil, 0.956 for EPA and AA, 0.959 for DHA.

A_s — The peak area of EPA or DHA or AA (methyl ester) in standard solution;

c_{si} —The concentration of C_{23:0} methyl ester internal standard in standard solution, mg/mL;

A_i —The peak area of C_{23:0} methyl ester internal standard in the test sample;

m —The corresponding mass of test sample in the final sample solution, g.

NOTE: One decimal digit of the calculated result is recommended.

9 Limit of determination and recovery

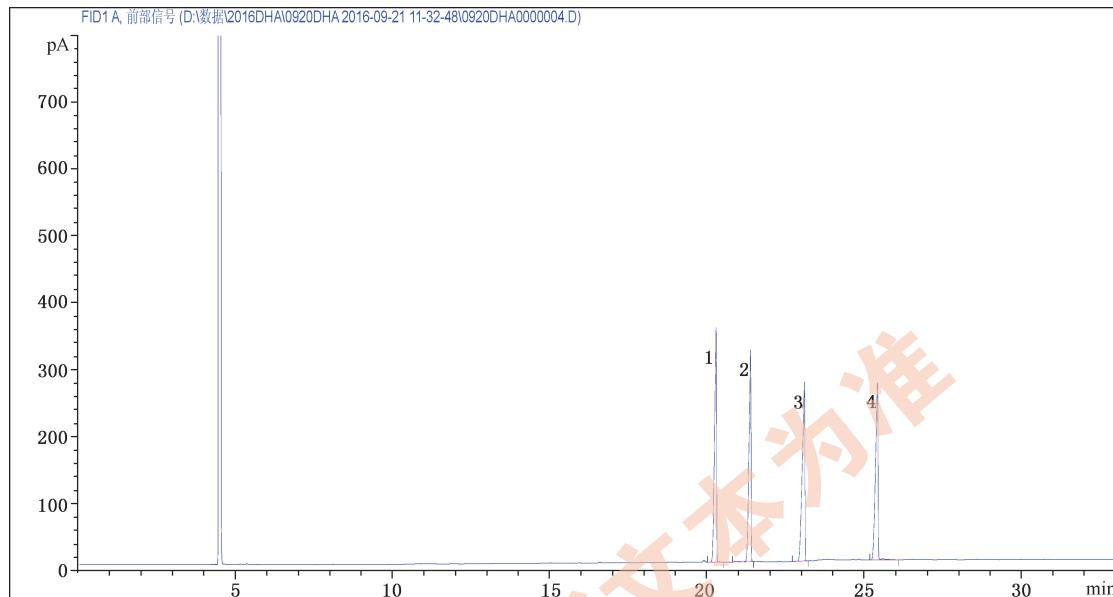
9.1 limit of quantitation

The limit of determination of both EPA and DHA and AA is 1.0 mg/g.

9.2 Recovery

The recovery of EPA and DHA and AA fortified in every kind of encapsulated composite fish oil products was between 97.1% to 102% and 97.2% to 102% and 97.0% to 103%. See annex B.

Annex A
(informative)
GC chromatogram of EPA and DHA and AA standards



Description of indexing serial number;

1—AA methyl ester;

2—EPA methyl ester;

3—IS, C_{23:0} methyl ester;

4—DHA methyl ester.

Figure A.1 GC-FID chromatogram of AA and EPA and DHA methyl ester standard

Annex B
(informative)
Recovery of EPA and DHA and AA

Table B.1 The recovery of EPA and DHA and AA

Sample name	Spiked level mg/g	Recovery/%		Spiked level mg/g	Recovery/%
		EPA	DHA		
Deep Sea Salmon Fish Oil capsules	50.0	97.4~100	97.6~101	10.0	97.6~102
	100	98.1~99.6	97.8~101	50.0	97.5~102
	200	98.8~100	98.7~101	100	98.4~101
Fish Oil DHA Extract	50.0	98.0~101	97.5~101	10.0	98.2~102
	100	99.2~101	98.0~100	50.0	98.0~102
	200	99.1~101	99.6~101	100	99.0~101
Fish Oil EPA Extract	50.0	97.1~102	98.0~102	10.0	97.4~103
	100	98.8~101	98.2~101	50.0	97.7~101
	200	99.1~101	98.8~101	100	98.1~101
Tuna Oil	50.0	98.3~101	97.6~101	10.0	97.4~102
	100	98.7~101	98.3~101	50.0	99.0~102
	200	99.1~101	98.5~100	100	99.8~102
Salmon Fish Oil	50.0	98.2~102	97.2~102	10.0	97.0~102
	100	98.5~101	98.5~101	50.0	98.0~101
	200	98.8~101	98.8~101	100	98.2~101
Salmon Fish Oil	50.0	98.0~102	98.0~103	10.0	97.5~101
	100	98.2~101	98.9~101	50.0	98.0~102
	200	99.1~101	99.3~101	100	98.2~100
Olive Oil	10	97.6~101	97.8~104	10	97.1~102
	50.0	97.3~102	97.8~103	50.0	99.8~103
	100	97.6~101	98.5~102	100	98.7~102
	200	98.8~101	98.6~101	200	98.7~101

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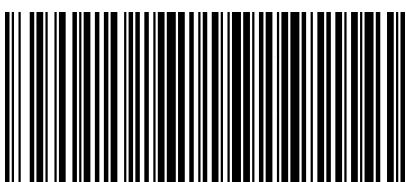
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