SN

中华人民共和国出入境检验检疫行业标准

SN/T 2922—2011

出口食品中 EPA 和 DHA 的测定 气相色谱法

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

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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国广东出入境检验检疫局。

本标准主要起草人：陈捷、叶弘毅、陈文锐、谢守新、林海丹。

出口食品中 EPA 和 DHA 的测定 气相色谱法

1 范围

本标准规定了出口鱼油和鱼油复合胶囊制品中顺式-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)的气相色谱测定方法。

本标准适用于鱼油和鱼油复合胶囊制品中 EPA、DHA 含量的测定。

2 方法提要

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

3 试剂和材料

除另有规定外,试剂均为分析纯,水为去离子水。

3.1 异辛烷。

3.2 甲醇。

3.3 氢氧化钠。

3.4 氯化钠。

3.5 三氟化硼甲醇溶液:BF_3,12%三氟化硼甲醇溶液。

3.6 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。

3.7 饱和氯化钠溶液:36 g 氯化钠,溶于 100 mL 水中。

3.8 标准物质

3.8.1 二十三酸甲酯:CAS 编号 2433-97-8,纯度≥99.0%,内标物。

3.8.2 顺式-5,8,11,14,17-二十碳五烯酸甲酯:CAS 编号 2734-47-6,纯度≥99.0%。

3.8.3 顺式-4,7,10,13,16,19-二十二碳六烯酸甲酯:CAS 编号 301-01-9,纯度≥99.0%。

3.9 标准溶液配制

3.9.1 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。

3.9.2 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 10.0 mg/mL 的标准储备液。

3.9.3 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 10.0 mg/mL 的标准储备液。

注:上述标准溶液应于 −18 ℃冰箱中保存,保存期六个月。

4 仪器和设备

4.1 气相色谱仪:配有火焰离子化(FID)检测器。

4.2 天平：感量 0.000 1 g。

4.3 涡旋振荡器。

4.4 恒温箱。

4.5 氮气吹干仪。

4.6 顶空进样瓶或玻璃试管：25 mL，带聚四氟乙烯垫旋盖。

4.7 玻璃刻度试管：5.0 mL，具塞。

5 测定步骤

5.1 准确移取 2.0 mL 二十三酸甲酯(3.9.1)内标液(相当于内标物 2.0 mg)于顶空进样瓶中，用氮气吹干，待用。如果不即时使用，应冷冻储存。

5.2 称取试样(胶囊内含物)0.02 g(精确至 0.000 1 g)于含有内标的顶空进样瓶(5.1)中，加 1.5 mL 0.5 mol/L 氢氧化钠甲醇溶液(3.6)，充入氮气，加盖密封，涡旋振荡混匀，100 ℃下加热 5 min，冷却，加 2 mL 三氟化硼甲醇溶液(3.5)，充入氮气，加盖密封，涡旋振荡混匀，100 ℃下加热 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(3.7)，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，通氮气浓缩并定容至 1.0 mL，待测。

5.3 标准系列工作液配制：准确移取 EPA 甲酯(3.9.2)和 DHA 甲酯(3.9.3)适量(分别相当于 EPA 甲酯和 DHA 甲酯 0.02 mg、0.5 mg、1.0 mg、2.0 mg、4.0 mg、10 mg)，按 5.2 操作。配制成标准系列工作液，EPA 甲酯和 DHA 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，内标浓度为 2.0 mg/mL。

5.4 测定

5.4.1 气相色谱条件

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

5.4.1.2 升温程序：150 ℃(2 min)$\xrightarrow{3\ ℃/min}$200 ℃$\xrightarrow{2\ ℃/min}$220 ℃(2 min)$\xrightarrow{25\ ℃/min}$230 ℃(2 min)。

5.4.1.3 进样口温度：250 ℃。

5.4.1.4 检测器温度：280 ℃。

5.4.1.5 载气：氢气(纯度 99.999%)，流量 2.0 mL/min。

5.4.1.6 进样模式：分流进样，分流比 30∶1。

5.4.1.7 进样量：1.0 μL。

5.4.2 气相色谱测定

内标法定量：标准工作溶液(5.3)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，EPA 甲酯的保留时间约为 22.4 min，DHA 甲酯的保留时间约为 27.9 min。标准品的色谱图参见图 A.1。

6 结果计算和表述

用色谱数据处理机或按式(1)计算试样中 EPA 或 DHA 的含量。

$$X_i = \frac{c_i \times V}{m \times k} \qquad \cdots\cdots(1)$$

式中：

X_i ——试样中 EPA 或 DHA 含量，单位为毫克每克(mg/g)；

c_i ——从标准工作曲线得到的 EPA 甲酯或 DHA 甲酯溶液浓度，单位为毫克每毫升(mg/mL)；

V ——最终样液的定容体积，单位为毫升(mL)；

m ——最终样液所代表试样量，单位为克(g)；

k ——1.04 或 1.05，1.04 为 DHA 甲酯换算为 DHA 的换算因子，1.05 为 EPA 甲酯换算为 EPA 的换算因子。

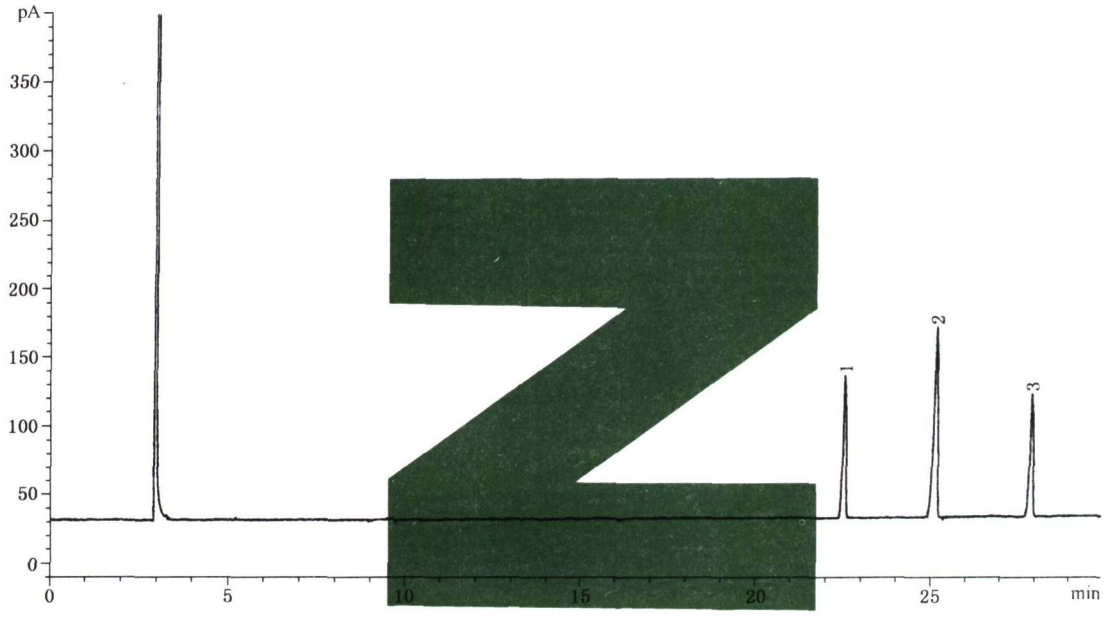
注：计算结果应表示到小数点后一位。

7 测定低限、回收率

EPA、DHA 的测定低限均为 1.0 mg/g。

在各种鱼油胶囊中，EPA、DHA 的回收率范围分别为 93.2%～104%和 93.7%～104%，参见表 B.1。

附 录 A
（资料性附录）
EPA 甲酯和 DHA 甲酯的标准品气相色谱图



1——EPA 甲酯；
2——内标物；
3——DHA 甲酯。

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

附 录 B
（资料性附录）
EPA 和 DHA 的回收率数据

表 B.1 EPA 和 DHA 的回收率

样品名称	EPA		DHA	
	添加水平 mg/g	回收率范围 %	添加水平 mg/g	回收率范围 %
橄榄油	1.00	95.4～103	1.00	95.9～101
	50.0	99.6～101	50.0	98.5～101
	100	99.2～101	100	99.6～103
	200	99.2～101	200	98.8～100
深海鱼油复合胶囊	50.0	96.5～100	50.0	96.1～102
	100	96.0～102	100	96.7～103
	200	97.9～101	200	98.3～101
海藻 DHA 软胶囊	50.0	96.5～103	50.0	94.0～97.4
	100	98.4～101	100	99.0～102
	200	95.4～103	200	97.6～101
鱼油银杏复合胶囊	50.0	95.4～101	50.0	95.2～101
	100	95.0～102	100	95.4～101
	200	96.1～101	200	97.6～100
鱼油纳豆复合胶囊	50.0	95.7～103	50.0	96.4～104
	100	96.4～104	100	95.2～101
	200	98.4～103	200	96.6～101
鱼油提取物胶囊	1.00	95.4～103	1.00	96.1～101
	50.0	99.3～102	50.0	98.4～101
	100	99.5～101	100	98.6～102
芦荟提取物胶囊	50.0	93.2～98.3	50.0	93.7～99.1
	100	93.6～99.5	100	95.2～100
	200	95.7～99.1	200	98.6～100
花生油	50.0	97.6～101	50.0	97.6～102
	100	96.4～104	100	95.2～101
	200	96.4～100	200	95.6～101
棕榈油	50.0	98.2～103	50.0	98.4～101
	100	98.3～101	100	96.8～99.8
	200	99.6～101	200	99.5～101

Foreword

This standard was drafted in accordance with the GB/T 1.1—2009.

This standard was proposed by and is under the charged of the Certification and Accreditation Administration of the people's Republic of China.

This standard was drafted by Guangdong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Chen Jie, Ye Hongyi, Chen Wenrui, Xie Shouxin, Lin Haidan.

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

1 Scope

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

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

2 Principle

Fatty acids include EPA and DHA of oils are derivatized to methyl esters by reacting with methanolic NaOH and BF_3 in methanol. EPA and DHA are determined by gas chromatograph with flame ionization detector and quantitatively determined using internal standard method.

3 Reagents and materials

Unless otherwise specified,all regents used should be analytical grade,"water"is distilled water.

3.1 Isooctane.

3.2 Methanol.

3.3 Sodium hydroxide.

3.4 Sodium chloride.

3.5 Boron trifluoride:BF_3,12% in methanol.

3.6 Alcoholic sodium hydroxide:0.5 mol/L. Dissolve 2.0 g NaOH in methanol and dilute to 100 mL with methanol.

3.7 Sodium chloride:Saturated solution. Dissolve 36 g NaCl in 100 mL water.

3.8 Reference materials

3.8.1 $C_{23:0}$ methyl ester: CAS No. 2433-97-8. purity≥99%.

3.8.2 all-*cis*-5,8,11,14,17-eicosapentaenoic methyl ester: CAS No. 2734-47-6. purity≥99%.

3.8.3 all-*cis*-4,7,10,13,16,19-docosahexaenoic methyl ester: CAS No. 301-01-9. purity≥99%.

3.9 Preparation of standards

3.9.1 $C_{23:0}$ 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.

3.9.2 EPA standard stock solution

Accurately weigh adequate amount of $C_{20:0}$ methyl ester standard (accurate to 0.000 1 g), dissolve with isooctane and prepare a solution of 10.00 mg/mL as standard stock solution.

3.9.3 DHA standard stock solution

Accurately weigh adequate amount of $C_{22:0}$ methyl ester standard (accurate to 0.000 1 g), dissolve with isooctane and prepare a solution of 10.00 mg/mL as standard stock solution.

Note: All the standard solutions should be stored at −18 ℃ in a refrigerator. They can be kept for six months.

4 Apparatus and equipment

4.1 Gas chromatograph: With flame ionization detector, capillary column injection system.

4.2 Analytical balance: 0.000 1 g.

4.3 Vortex mixer.

4.4 Oven.

4.5 Nitrogen evaporator.

4.6 Head-space injection vials or glass tubes: 25 mL, with leak-tight, teflon-lined screw caps.

4.7 Glass tubes with scale: 5 mL, with stoppers.

5 Procedures

5.1 Accurately pipet 2.0 mL of $C_{23:0}$ methyl ester internal standard solution(3.9.1) into vials and evaporate solvent in gentle stream of N_2. Store vials in freezer if not to be used immediately.

5.2 Accurately weigh 0.020 g(accurate to 0.000 1 g)oil into a vial containing methyl ester IS(5.1). Add 1.5 mL 0.5 mol/L methanolic NaOH(3.6),blanket with nitrogen,cap,mix on vortex mixer,and heat 5 min at 100 ℃. Cool,add 2 mL BF_3 in methanol(3.5),blanket with N_2,cap tightly,mix,and heat 30 min at 100 ℃. Cool mixture to 30 ℃～40 ℃,add 1 mL isooctane,and shake vigorously for 30 s while still warm. Immediately add 5 mL saturated NaCl solution(3.7),and agitate thoroughly. Cool to room temperature. When isooctane layer separates from aqueous lower phase,transfer isooctane layer to a clean glass tube,blanket with N_2,and cap. Extract aqueous lower phase a second time with an additional 1 mL isooctane. Combine isooctane extracts and concentrate to 1 mL in stream of dry N_2.

5.3 Preparation of standard working solutions

Accurately pipe a serials amount of EPA and DHA standard stock solutions(3.9.2,3.9.3),the amount of EPA and DHA are 0.02 mg,0.5 mg,1.0 mg,2.0 mg,4.0 mg,10 mg,respectively. Then operate as described in 5.2 to make standard working solution mixtures of EPA and DHA,which concentration are 0.02 mg/mL,0.5 mg/mL,1.0 mg/mL,2.0 mg/mL,4.0 mg/mL,10.0 mg/mL,respectively. The concentration of IS is 2 mg/mL.

5.4 Determination

5.4.1 GC operating conditions

5.4.1.1 GC column:Capillary column,DB-23,60 m × 0.25 mm(i.d.) × 0.15 μm or equivalent column.

5.4.1.2 Oven temperature programme:

150 ℃(hold 2 min) $\xrightarrow{3\ ℃/min}$ 200 ℃ $\xrightarrow{2\ ℃/min}$ 220 ℃(hold 2 min) $\xrightarrow{25\ ℃/min}$ 230 ℃(hold 2 min).

5.4.1.3 Injection port temperature:250 ℃.

5.4.1.4 Detector temperature:280 ℃.

5.4.1.5 Carrier gas:Hydrogen,purity 99.999%;Fow rate:2.0 mL/min.

5.4.1.6 Injection mode:Split mode preferred at split ratio of 30∶1.

5.4.1.7 Injection volumn:1 μL.

5.4.2 GC determination

Internal standard method quantitation: Inject standard working solutions and sample solutions alternatively. The calibration curve is made as follows: The area ratio of target peak to the IS is used as yaxis, 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 and DHA 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 EPA and DHA are 22.4 min and 27.9 min, respectively. The GC chromatogram of EPA and DHA standard see figure A.1.

6 Calculation and expression of result

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

$$X_i = \frac{c_i \times V}{m \times k} \qquad \cdots\cdots(1)$$

Where

X_i —the content of EPA or DHA in the test sample, mg/g;

c_i —the concentration of EPA or DHA(methyl ester)in the sample solution calculated from the calibration curve, mg/mL;

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

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

k —factor necessary to express result as mg fatty acid/g oil, 1.05 for EPA, 1.04 for DHA.

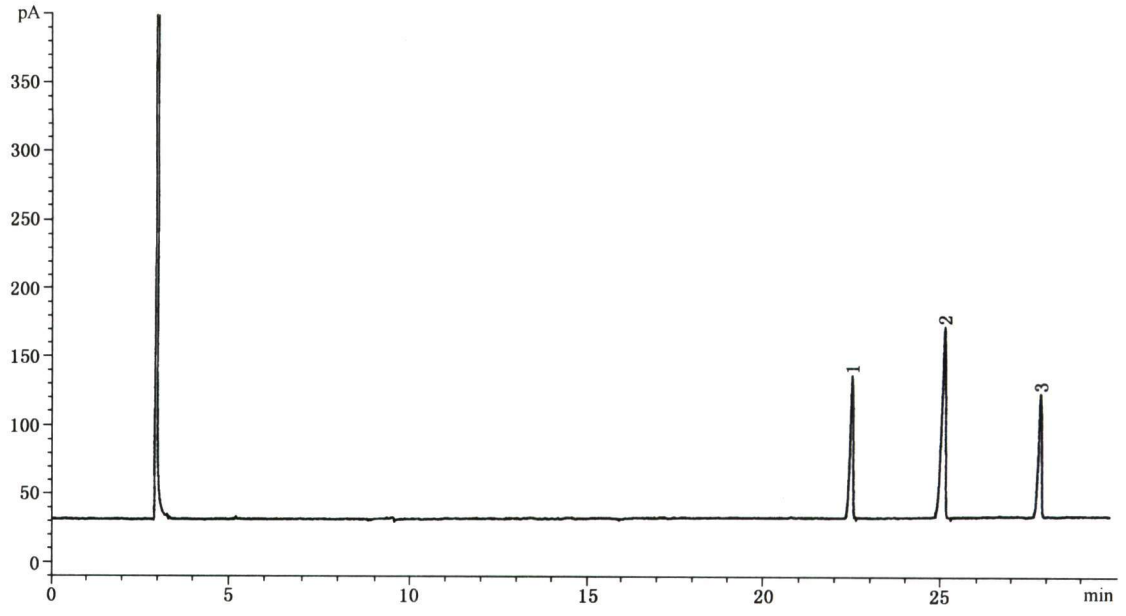
Note: One decimal digit of the calculated result is recommended.

7 Limit of determination and recovery

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

The recovery of both EPA and DHA fortified in every kind of encapsulated composite fish oil products was between 93.2%～104% and 93.7%～104%. See table B.1.

Annex A
(Informative)
GC chromatogram of EPA and DHA standards



1—EPA methyl ester;

2—IS, $C_{23:0}$ methyl ester;

3—DHA methyl ester.

Figure A. 1—GC-FID chromatogram of EPA and DHA methyl ester standard(1 mg/mL)

Annex B
(Informative)
Recovery of EPA and DHA

Table B. 1—The recovery of EPA and DHA

Sample	EPA		DHA	
	Fortified concentration mg/g	Recovery %	Fortified concentration mg/g	Recovery %
Olive oil	1.00	95.4～103	1.00	95.9～101
	50.0	99.6～101	50.0	98.5～101
	100	99.2～101	100	99.6～103
	200	99.2～101	200	98.8～100
Deep sea fish oil capsule	50.0	96.5～100	50.0	96.1～102
	100	96.0～102	100	96.7～103
	200	97.9～101	200	98.3～101
Alga DHA soft gel capsule	50.0	96.5～103	50.0	94.0～97.4
	100	98.4～101	100	99.0～102
	200	95.4～103	200	97.6～101
Gingko and fish oil soft gel capsule	50.0	95.4～101	50.0	95.2～101
	100	95.0～102	100	95.4～101
	200	96.1～101	200	97.6～100
Fish oil and soybean capsule	50.0	95.7～103	50.0	96.4～104
	100	96.4～104	100	95.2～101
	200	98.4～103	200	96.6～101
Fish oil extracts capsule	1.00	95.4～103	1.00	96.1～101
	50.0	99.3～102	50.0	98.4～101
	100	99.5～101	100	98.6～102
Aloe extracts capsule	50.0	93.2～98.3	50.0	93.7～99.1
	100	93.6～99.5	100	95.2～100
	200	95.7～99.1	200	98.6～100
Peanut oil	50.0	97.6～101	50.0	97.6～102
	100	96.4～104	100	95.2～101
	200	96.4～100	200	95.6～101

Table B. 1 (continued)

Sample	EPA		DHA	
	Fortified concentration mg/g	Recovery %	Fortified concentration mg/g	Recovery %
Palm oil	50. 0	98. 2～103	50. 0	98. 4～101
	100	98. 3～101	100	96. 8～99. 8
	200	99. 6～101	200	99. 5～101

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