

Cod Liver Oil

DEFINITION
Cod Liver Oil is the partially destearinated fixed oil obtained from fresh livers of *Gadus morrhua* L. and other species of Family Gadidae. Cod Liver Oil contains, in each gram, NLT 180 µg and NMT 750 µg of vitamin A and NLT 1.5 µg and NMT 6.25 µg of vitamin D. Cod Liver Oil may be flavored by the addition of NMT 1% of a suitable flavor or a mixture of flavors. A suitable antioxidant may be added.

IDENTIFICATION

• **A. PRESENCE OF VITAMIN A**
Sample solution: 25 mg/mL of Cod Liver Oil in [chloroform](#)
Analysis: To 1 mL of the *Sample solution* add 10 mL of [antimony trichloride TS](#).
Acceptance criteria: A blue color results immediately.
Change to read:

• **B. FATTY ACID PROFILE**
Antioxidant solution: 0.05 mg/mL of [butylated hydroxytoluene](#) in [hexanes](#)
System suitability solution: Prepare a mixture containing equal amounts of methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate in *Antioxidant solution*.
Standard stock solution: 45 mg/mL of [USP Cod Liver Oil RS](#) in *Antioxidant solution*
Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a glass tube,¹ and evaporate with a gentle stream of nitrogen. Add 1.5 mL of a 2% solution of [sodium hydroxide](#) in [methanol](#), cap tightly with a polytetrafluoroethylene-lined cap, mix, and heat in a water bath for 7 min. Cool, add 2 mL of a 120-mg/mL solution of boron trichloride in [methanol](#), cover with nitrogen, cap tightly, mix, and heat in a water bath for 30 min. Cool to 40°–50°, add 1 mL of [isooctane](#), cap, and mix in a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated [sodium chloride](#) solution, cover with nitrogen, cap, and mix in a vortex mixer or shake thoroughly for at least 15 s. Allow the upper layer to become clear, and transfer to a separate tube. Shake the methanol layer once more with 1 mL of [isooctane](#), and combine the isooctane extracts. Wash the combined extracts twice with 1 mL of [water](#), and dry over anhydrous [sodium sulfate](#).
Sample solution: Prepare as directed for the *Standard solution*, except replace [USP Cod Liver Oil RS](#) with Cod Liver Oil.
Chromatographic system
(See [Chromatography \(621\)](#), [System Suitability](#).)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-µm film of phase [G16](#)
Temperatures
Injector port: 250°
Detector: 280°
Column: See [Table 1](#).

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	1	225	20

Carrier gas: Helium
▲Flow rate: 1.0 mL/min▲ (USP 1-Dec-2020)

Injection volume: 1 µL

Injection type: Split; split ratio, 200:1

▲[NOTE—The use of a deactivated split liner (4.0-mm i.d.) with glass wool is recommended.]▲ (USP 1-DEC-2020)

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.3 between methyl oleate and methyl *cis*-vaccinate, and that between methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement, *Standard solution*

Theoretical area percentages: 24.4 ± 1 for methyl palmitate, 24.8 ± 1 for methyl stearate, 25.2 ± 1 for methyl arachidate, and 25.6 ± 1 for methyl behenate, *System suitability solution*

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram supplied with [USP Cod Liver Oil RS](#). Identify the retention times of the relevant fatty acid methyl esters by comparing the chromatogram of the *Standard solution* with the reference chromatogram supplied with [USP Cod Liver Oil RS](#).

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution*.

Determine the number of fatty acid methyl esters in the *Sample solution*. The number of fatty acid methyl ester peaks exceeding 0.05% of the total area of fatty acid methyl esters is at least 24, and the 24 largest peaks of the methyl esters account for more than 90% of the total area. (These correspond to the following, in common elution order: 14:0, 15:0, 16:0, 16:1 *n*-7, 16:4 *n*-1, 18:0, 18:1 *n*-9, 18:1 *n*-7, 18:2 *n*-6, 18:3 *n*-3, 18:4 *n*-3, 20:1 *n*-11, 20:1 *n*-9, 20:1 *n*-7, 20:2 *n*-6, 20:4 *n*-6, 20:3 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:1 *n*-11, 22:1 *n*-9, 21:5 *n*-3, 22:5 *n*-3, and 22:6 *n*-3.)

Calculate the area percentage for each fatty acid methyl ester in the portion of Cod Liver Oil taken:

$$\text{Result} = (r_A/r_B) \times 100$$

r_A = peak area of each individual fatty acid methyl ester in the *Sample solution*

r_B = total area from all peaks, except the solvent peak and butylated hydroxytoluene, in the *Sample solution*

Acceptance criteria: The *Sample solution* meets the limits described in [Table 2](#).

Table 2

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Saturated fatty acids			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
Monounsaturated fatty acids			
Palmitoleic acid	16:1 <i>n</i> -7	4.5	11.5
<i>cis</i> -Vaccenic acid	18:1 <i>n</i> -7	2.0	7.0
Oleic acid	18:1 <i>n</i> -9	12.0	21.0
Gadoleic acid	20:1 <i>n</i> -11	1.0	5.5
Gondoic acid	20:1 <i>n</i> -9	5.0	17.0
Erucic acid	22:1 <i>n</i> -9	0	1.5