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Krill Oil

DEFINITION

Krill Oil is the fixed oil extracted from Antarctic krill (*Euphausia superba* Dana) biomass using appropriate food-grade organic solvents. Krill Oil contains NLT 30% (w/w) and NMT 59% (w/w) of total phospholipids, of which 60%–96% are phosphatidylcholine, 1-lysophosphatidylcholine, and 2-lysophosphatidylcholine. It contains NLT 10% (w/w) of eicosapentaenoic acid (EPA) and NLT 5.0% (w/w) of docosahexaenoic acid (DHA) mostly in the form of phospholipids. It also contains NLT 0.005% of astaxanthin.

IDENTIFICATION

• **A. FATTY ACID PROFILE**

Antioxidant solution, System suitability solution 1, and Chromatographic system: Proceed as directed in [Fats and Fixed Oils \(401\), Procedures, Omega-3 Fatty Acids Determination and Profile](#).

Standard solution: Prepare as directed in [Fats and Fixed Oils \(401\), Procedures, Omega-3 Fatty Acids Determination and Profile, Content of EPA and DHA, Test Solution 1](#), except use 250 mg of [USP Krill Oil RS](#).

Sample solution: Prepare as directed in the *Standard solution*, except replace [USP Krill Oil RS](#) with Krill Oil.

System suitability

Samples: *System suitability solution 1* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the methyl oleate and methyl *cis*-vaccinate peaks, *Standard solution*

Theoretical area percentages: Meets the requirements for *System suitability solution 1*

Chromatogram similarity: The chromatogram obtained from the *Standard solution* is similar to the reference chromatogram provided with the lot of [USP Krill Oil RS](#) being used.

Analysis

Sample: *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* and the USP reference chromatogram.

Calculate the area percentage for each fatty acid as methyl esters in the portion of Krill Oil taken:

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

r_A = peak area of each individual fatty acid from the *Sample solution*

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

Acceptance criteria: The fatty acids obtained from the *Sample solution* meet the limit requirements in [Table 1](#).

Table 1

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Saturated fatty acids			
Myristic acid	14:0	5.0	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid:myristic acid ratio	16:0/14:0	1.6	3.6
Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	2.5	12.0

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
cis-Vaccenic acid	18:1 n-7	4.7	8.0
Oleic acid	18:1 n-9	6.0	14.5
Eicosenoic acid	20:1 n-9	0.0	2.0
Erucic acid	22:1 n-9	0.0	1.5
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	0.0	3.0
Eicosapentaenoic acid	20:5 n-3	14.0	28.0
Docosapentaenoic acid	22:5 n-3	0.0	0.7
Docosahexaenoic acid	22:6 n-3	7.1	15.7

• B. PHOSPHOLIPID PROFILE

Solution A, Line shape standard (¹H), Sensitivity standard (¹H), Sensitivity standard (³¹P), Internal standard, Sample solution, Standard solution, Instrumental conditions, System suitability, and Analysis: Proceed as directed in the test for *Content of Total Phospholipids*.

Acceptance criteria: The *Sample solution* contains all of the following phospholipids: phosphatidylcholine, lysophosphatidylcholine, and phosphatidylethanolamine. The sum of phosphatidylcholine, 1-lysophosphatidylcholine, and 2-lysophosphatidylcholine is 60%–96%.

COMPOSITION

• CONTENT OF EPA AND DHA

Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, System suitability solution 1, and

Chromatographic system: Proceed as directed in [Fats and Fixed Oils \(401\)](#), [Procedures, Omega-3 Fatty Acids Determination and Profile](#).

Test solution 1: Prepare as directed in [Fats and Fixed Oils \(401\)](#), [Procedures, Omega-3 Fatty Acids Determination and Profile](#), [Content of EPA and DHA, Test Solution 1](#), except use 250 mg of Krill Oil.

Test solution 2: Prepare as directed in [Fats and Fixed Oils \(401\)](#), [Procedures, Omega-3 Fatty Acids Determination and Profile](#), [Content of EPA and DHA, Test Solution 2](#), except use 250 mg of Krill Oil.

Analysis: Proceed as directed in [Fats and Fixed Oils \(401\)](#), [Procedures, Omega-3 Fatty Acids Determination and Profile](#), [Content of EPA and DHA, Analysis \(for triglycerides\)](#).

Acceptance criteria: NLT 10.0% (w/w) of EPA and NLT 5.0% (w/w) of DHA

• CONTENT OF TOTAL PHOSPHOLIPIDS

(See [Nuclear Magnetic Resonance Spectroscopy \(761\)](#), [Qualitative and Quantitative NMR Analysis](#).)

[NOTE—All deuterated solvents used in this method should be NLT 99.8 atom % D. Whenever water is used in this method, it should be of sufficient quality to ensure that no trace metals or other contaminants that may affect the analysis are present.]

Solution A: 0.2 M [EDTA](#), adjusted with a 1 M [cesium carbonate](#) solution to a pH of 7.2–7.5. Document the final pH and the amount of 1 M [cesium carbonate](#) solution necessary to attain the desired pH. [NOTE—Use cesium carbonate of a sufficient grade for trace metals analysis.]

Line shape standard (¹H): 1% [chloroform](#) in acetone-d₆

Sensitivity standard (¹H): 0.1% [ethylbenzene](#) in chloroform-d

Sensitivity standard (³¹P): 0.0485 M [triphenyl phosphate](#) in [acetone-d₆](#)

Internal standard: Use a suitable [triphenyl phosphate](#) NMR analytical standard with purity of NLT 99.0%.

Sample solution: [NOTE—NMR solvents containing [tetramethylsilane](#) (TMS) are readily available. If the solvents used do not contain TMS, it must be added to the *Sample solution* at an approximate concentration of 0.05% (v/v) for use as a chemical shift scale reference.] Transfer 300–350 mg of Krill Oil to a 5-mL sealable glass vial. Add 25.0 mg of the *Internal standard* to the vial. Add 1 mL each of [deuterated chloroform \(chloroform-d\)](#) and [deuterated methanol \(methanol-d₄\)](#) of a grade suitable for NMR analysis to the vial to dissolve the sample.

Once dissolution is complete, add 1 mL of *Solution A*, seal the vial, and shake the solution for 10–20 min, and then centrifuge the contents of the vial. Transfer the lower organic phase to an appropriate NMR tube. It is critical to collect the entire organic phase and transfer it to the NMR tube. It may be unavoidable to also transfer small amounts of the aqueous phase when collecting the organic phase in the NMR tube. This is acceptable practice, so long as the aqueous phase remains completely separated and atop the organic phase in the NMR tube. The entire amount of aqueous phase must be above the probe's radio frequency (RF) coil (outside the analysis area of the tube). Should the organic phase contain undissolved materials, they must remain suspended at the aqueous-organic interface and be in the analysis area of the tube as well. The organic phase must be free of bubbles and suspended materials that may interfere with NMR data acquisition.