Krill Oil Capsules

DEFINITION

Change to read:

▲Krill Oil Capsules contain NLT 95.0% of the labeled amount of Krill Oil calculated through the content of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and total phospholipids.▲ (USP 1-May-2019)

IDENTIFICATION

Change to read:

• **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, Test solution 1*, except use 250 mg of USP Krill Oil RS.

Sample solution: Using the portion of oil from NLT 10 Capsules, prepare as directed in *Fats and Fixed Oils ⟨401⟩, Procedures, Omega-3 Fatty Acids Determination and Profile, Test solution 1*.

System suitability

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

Suitability requirements

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

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

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

Analysis

Sample: *Standard solution* and *Sample solution*

Identify the retention times of the peaks corresponding to the relevant fatty acid methyl esters by comparing the chromatogram of the *Standard solution* to the reference chromatogram provided with the lot of USP Krill Oil RS used.

Calculate the area percentage for each fatty acid as methyl esters in the portion of oil taken from the Capsules:

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

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: See *Table 1*.

Table 1

Fatty Acid	Short-Hand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Saturated fatty acids			
Myristic acid	14:0	▲5.0▲ (USP 1-May-2019)	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid:myristic acid ratio	16:0/14:0	1.6	▲3.6▲ (USP 1-May-2019)
Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	2.5	▲12.0▲ (USP 1-May-2019)
cis-Vaccenic acid	18:1 n-7	4.7	8.0
Oleic acid	18:1 n-9	▲6.0▲ (USP 1-May-2019)	14.5
Eicosenic acid	20:1 n-9	0.0	2.0

Fatty Acid	Short-Hand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	0.0	3.0
Eicosapentaenoic acid	20:5 n-3	14.0	▲28.0▲ (USP 1-May-2019)
Docosapentaenoic acid	22:5 n-3	0.0	0.7
Docosahexaenoic acid	22:6 n-3	7.1	15.7

Change to read:

• **B. Phospholipid Profile**

Solution A, ▲▲ (USP 1-May-2019) **Internal standard,** ▲**Sample stock solution,**▲ (USP 1-May-2019) **Sample solution, Standard solution, Instrumental conditions, System suitability,** and **Analysis:** Proceed as directed in the test for *Content of Total Phospholipids* in *Strength*.

Acceptance criteria: ▲The *Sample solution* shows ^{31}P nuclear magnetic resonance (NMR) spectra similar to those obtained with USP Krill Oil RS. The main signal in the ^{31}P NMR is due to phosphatidylcholine (PC); a signal due to phosphatidylcholine ether has an intensity of about 10% of that due to PC; and the second signal in intensity is due to 2-lysophosphatidylcholine (2-LPC). Minor signals due to phosphatidylethanolamine (PE), *N*-acylphosphatidylethanolamine (NAPE), lysophosphatidylethanolamine (LPE), and 1-lysophosphatidylcholine (1-LPC) among others are also observed.▲ (USP 1-May-2019)

STRENGTH

Delete the following:

▲• **Content of Krill Oil**

Analysis: Weigh NLT 10 Capsules in a tared weighing bottle, and carefully open the Capsules, without loss of shell material. Transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of 2,2,4-trimethylpentane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the 2,2,4-trimethylpentane is completely evaporated. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule.

Acceptance criteria: 95.0%–105.0%▲ (USP 1-May-2019)

Change to read:

• **Content of EPA and DHA**

(See *Fats and Fixed Oils ⟨401⟩, Procedures, Omega-3 Fatty Acids Determination and Profile*).

Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, System suitability solution 1, and **Chromatographic system:** Proceed as directed in the chapter.

Test solution 1: ▲Weigh NLT 10 Capsules in a tared weighing bottle, and carefully open the Capsules without loss of shell material. Transfer the combined contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of acetone. Discard the washings, and allow the empty Capsules to dry in a current of air until the acetone is completely evaporated. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average fill weight per Capsule. Take 250 mg of the combined Capsule contents and▲ (USP 1-May-2019) proceed as directed in *Fats and Fixed Oils ⟨401⟩, Procedures, Omega-3 Fatty Acids Determination and Profile, Test solution 1*.

Test solution 2: ▲▲ (USP 1-May-2019) Prepare as directed in *Fats and Fixed Oils ⟨401⟩, Procedures, Omega-3 Fatty Acids Determination and Profile, Test solution 2*▲using 250 mg of the combined Capsule contents.▲ (USP 1-May-2019)

Analysis: Proceed as directed in *Fats and Fixed Oils ⟨401⟩, Procedures, Omega-3 Fatty Acids Determination and Profile, Analysis (for triglycerides)*.

▲Calculate the percentage of the labeled amounts of EPA and DHA in the portion of Capsules taken:

$$\text{Result} = A \times W_{Av} \times 100/L$$

A = content of EPA or DHA in the portion of Capsules taken (mg/mg)

W_{Av} = average fill weight (mg/Capsule)

L = label claim of EPA or DHA (mg/Capsule)▲ (USP 1-May-2019)

Acceptance criteria: ▲NLT 95.0% of the labeled amounts of EPA and DHA▲ (USP 1-May-2019)

Change to read:

• **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. ▲▲ (USP 1-May-2019)]

Solution A: 0.2 M ethylenediaminetetraacetic acid (EDTA) adjusted with a 1 M cesium carbonate solution to a pH of 7.2–7.5. ▲▲ (USP 1-May-2019)[Note—Use cesium carbonate of a sufficient grade for trace metals analysis.]

▲▲ (USP 1-May-2019)

Internal standard: Use a triphenyl phosphate nuclear magnetic resonance (NMR) reference standard with NLT 99% purity.

▲**Sample stock solution:** Transfer a number of Capsules (NLT 5) to a conical flask with stopper. Add 2 mL/Capsule of water and melt the gelatin shells at 50°–60°. Add 10 mL/Capsule of chloroform and methanol (2:1), insert the stopper and shake intensively for 20 min. Transfer to a separation funnel fitted with glass wool

Sample solution: Transfer an aliquot of the *Sample stock solution* equivalent to 300 mg of Krill Oil to a suitable sealable glass vial, and add about 25 mg of the *Internal standard* accurately weighed. Evaporate to dryness. Add 2 mL of deuterated chloroform (chloroform-d) containing 0.05% tetramethylsilane (TMS), 2 mL of deuterated methanol (methanol-d4), and 2 mL of *Solution A*. Seal the vial and vortex intensively for 30–60 s, shake for an additional 30 min on a shaking device, and centrifuge the contents of the vial. Pass the entire amount of the lower organic phase through a glass fiber filter, and collect the filtrate in the appropriate NMR tube.▲ (USP 1-May-2019)

Standard solution: ▲Transfer 300 mg of USP Krill Oil RS to a suitable sealable glass vial, and add about 25 mg of the *Internal standard* accurately weighed. Add 2 mL of methanol-d4, 2 mL of chloroform-d containing 0.05% TMS, and 2 mL of *Solution A*. Seal the vial and vortex intensively for 30–60 s, shake for an additional 30 min on a shaking device, and centrifuge the contents of the vial. Pass the entire amount of the lower organic phase through a glass fiber filter, and collect the filtrate in the appropriate NMR tube.▲ (USP 1-May-2019)

Instrumental conditions

Magnetic field strength: ▲NLT 7.05 Tesla (resonance frequencies of 121 MHz for ^{31}P or 300 MHz for ^{1}H)▲ (USP 1-May-2019)

Probe: Direct observe probe capable of tuning to the resonance frequency of ^{31}P (dependent on the specific magnetic field strength used) ▲at a temperature of 35°▲ (USP 1-May-2019)

Data collection: Use the parameters specified in *Table 2*. Use 90° pulses, and calibrate pulses before use according to the recommendations supplied by the instrument manufacturer.

Table 2

Parameter	^{31}P NMR Quantitative Measurement	▲▲ (USP 1-May-2019)
Pulse program	^{1}H-decoupled ^{31}P (inverse gated)	▲▲ (USP 1-May-2019)
Spectral width	50 ppm (25 to −25 ppm)	▲▲ (USP 1-May-2019)
Transmitter offset	Center of spectral width, 0 ppm	▲▲ (USP 1-May-2019)
Relaxation delay	5–15 s	▲▲ (USP 1-May-2019)
Acquisition time	1–6 s	▲▲ (USP 1-May-2019)
Size of data set	NLT 64k (32k with zero-filling)	▲▲ (USP 1-May-2019)

[NOTE—The acquisition time is dependent upon the dwell time and the number of data points collected. The number of scans acquired using a ▲7.05 Tesla magnet▲ (USP 1-May-2019) must be NLT 512.]

System suitability: ▲The *Standard solution* shows the ^{31}P NMR signal for triphenyl phosphate at −17.80 ppm, and the signal for phosphatidylcholine at −0.89 ppm. The signal-to-noise ratio for the phosphatidylcholine signal in the ^{31}P spectrum of the *Sample solution* obtained in the *Analysis* is NLT 2000. Using the baseline as a reference, determine the height of the phosphatidylcholine ether peak and draw a line parallel to the baseline at 30% of that total peak height (intensity). The valley between the peaks of phosphatidylcholine ether and phosphatidylcholine is below the line drawn.▲ (USP 1-May-2019)

Analysis: ▲Obtain the quantitative ^{31}P spectrum of the *Sample solution* and the *Standard solution* as directed in *Data collection*. Record the resulting spectra, and integrate the complete set of phospholipid peaks as identified by a comparison with the reference spectrum provided with USP Krill Oil RS. The integration region for each signal must extend ±0.05 ppm on either side of the ^{31}P signal.▲ (USP 1-May-2019)

Calculations: Use the following equations and molecular weights listed in *Table 3* to ▲calculate the content of the phospholipid of interest in the Capsules taken:▲ (USP 1-May-2019)

$$mmol_{IS} = (W_{IS} \times C_{IS})/(MW_{IS} \times 100)$$

$mmol_{IS}$ = millimoles of the *Internal standard* in the *Sample solution* (mmol)

W_{IS} = weight of the *Internal standard* added to the *Sample solution* (mg)

C_{IS} = purity value of the *Internal standard*, based on quantitative ^{31}P NMR analysis (% by weight)

MW_{IS} = molecular weight of the *Internal standard*, 326.28 g/mol (for triphenyl phosphate)

$$mmol_{PL} = (I_{PL} \times A_{IS} \times mmol_{IS})/(I_{IS} \times A_{PL})$$

$mmol_{PL}$ = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

I_{PL} = integrated area under the phospholipid signal of interest obtained from the spectrum of the *Sample solution*

A_{IS} = number of phosphorus atoms per molecule expected from the *Internal standard*, 1 (for triphenyl phosphate)

I_{IS} = integrated area under the *Internal standard* obtained from the spectrum of the *Sample solution*

A_{PL} = number of phosphorus atoms per molecule expected from the phospholipid of interest, 1 (for any phospholipid listed in Table 3)

$$▲C_{PL} = 1/n \times MW_{PL} \times mmol_{PL} \times V/a$$

C_{PL} = content of the phospholipid of interest in the Capsules taken (mg/Capsule)

n = number of Capsules used to prepare the *Sample stock solution*

MW_{PL} = molecular weight of the phospholipid of interest (mg/mmol, from Table 3)

$mmol_{PL}$ = millimoles of the phospholipid of interest in the *Sample solution* (mmol)

V = volume of volumetric flask used to prepare the *Sample stock solution* (mL)

a = volume of the aliquot of *Sample stock solution* used to prepare the *Sample solution* (mL)▲ (USP 1-May-2019)

Table 3

Component	Approximate Chemical Shift (ppm) in Reference to Triphenyl Phosphate	Molecular Weight (g/mol)
Triphenyl phosphate (*Internal standard*)	−17.8	—
Phosphatidylcholine, including ether (PC)	−0.89	791
1-Lysophosphatidylcholine (1-LPC)[a]	−0.48	534.5
2-Lysophosphatidylcholine (2-LPC)[a]	−0.4	534.5
Phosphatidylethanolamine (PE)	−0.24	770
N-Acylphosphatidylethanolamine (NAPE)	0	1032
Lysophosphatidylethanolamine (LPE)	0.25	492.5
Other	—	800

[a] Ability to resolve the signals of 1-LPC and 2-LPC will depend upon the applied magnetic field strength of the NMR spectrometer used for the test procedure.

▲Calculate the percentage of the labeled amount of total phospholipids per Capsule taken:

$$\text{Result} = \Sigma C_{PL} \times 100/L$$

C_{PL} = sum of the individual amounts of phospholipids of interest in the Capsules taken (mg/Capsule)

L = label claim of total phospholipids (mg/Capsule)▲ (USP 1-May-2019)

Acceptance criteria: ▲NLT 95.0% of the labeled amount of total phospholipids▲ (USP 1-May-2019)

Change to read:

• **Content of Astaxanthin**

[Note—Perform this analysis in subdued light using low-actinic glassware.]

Sample solution: 0.005 g/mL of Krill Oil in chloroform using the portion of oil from NLT 10 Capsules. [Note—If the solution is not clear, centrifuge it with an appropriate centrifuge to obtain a clear supernatant.]

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy ⟨857⟩.)

Analytical wavelength: 486 nm

Cell: 1 cm

Blank: Chloroform

Analysis

Sample: *Sample solution*

Calculate the percentage of astaxanthin in the portion of Krill Oil taken from the Capsules:

$$\text{Result} = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = coefficient of extinction ($E^{1\%}$) of pure astaxanthin in chloroform (100 mL · g^{-1} · cm^{-1}), 1692

Acceptance criteria: NLT ▲0.005%▲ (USP 1-May-2019)

PERFORMANCE TESTS

• **Disintegration and Dissolution** ⟨2040⟩, *Rupture Test for Soft Shell Capsules*: Meet the requirements

• **Weight Variation** ⟨2091⟩: Meet the requirements

CONTAMINANTS

• **Limit of Dioxins, Furans, and Polychlorinated Biphenyls**

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by Method 1613, Revision B of the Environmental Protection Agency (EPA). Determine the content of polychlorinated biphenyls (PCBs) by Method 1668, Revision A of the EPA.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2.0 pg/g of World Health Organization (WHO) toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs [non-ortho International Union of Pure and Applied Chemistry (IUPAC) congeners PCB-77, PCB-81, PCB-126, and PCB-169; and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189] is NMT 10.0 pg/g of WHO toxic equivalents.

• **Microbial Enumeration Tests** ⟨2021⟩: The total aerobic microbial count does not exceed 10^3 cfu/g, and the combined molds and yeasts count does not exceed 10^2 cfu/g.

• **Absence of Specified Microorganisms** ⟨2022⟩, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meet the requirements

SPECIFIC TESTS

• **Astaxanthin Esterification**

Standard solution A: 10 mg/mL of USP Astaxanthin Esters from *Haematococcus pluvialis* RS in acetone

Standard solution B: 10 mg/mL of USP Astaxanthin (Synthetic) RS in acetone

Sample solution: Using the portion of oil from NLT 10 Capsules, prepare a solution of 250 mg/mL in acetone.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel. [Note—Dry silica gel at 110° for 1 h before use.]

Application volume: 5 µL

Developing solvent system: Hexane and acetone (70:30)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about 15 cm of the length of the plate. Remove the plate from the chamber, and allow to dry.

Acceptance criteria: The principal spot from *Standard solution B*, located in the bottom half of the plate, is free astaxanthin. The *Sample solution* may exhibit a light, minor spot in the same location. The principal spots from *Standard solution A* are from monoesters (primary spot, located slightly above the middle of the plate) and diesters (secondary spot, located in the top third of the plate). The principal spot from the *Sample solution* should correspond in color and R_F value to the diester spot from *Standard solution A*. The secondary spot from the *Sample solution* should correspond in color and approximately the same R_F value to the monoester spot from *Standard solution A*. [Note—Slight differences in R_F values within monoester spots and within diester spots may exist because of different intensities.]

• **Fats and Fixed Oils** ⟨401⟩, *Procedures, Peroxide Value*: NMT 5.0 mEq peroxide/kg

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at room temperature. Protect from light.

Change to read:

• **Labeling:** The label states the amount of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and total phospholipids ▲in mg/Capsule.▲ (USP 1-May-2019)

Change to read:

• **USP Reference Standards** ⟨11⟩

USP Astaxanthin (Synthetic) RS

USP Astaxanthin Esters from *Haematococcus pluvialis* RS

▲▲ (USP 1-May-2019)

USP Krill Oil RS

▲▲ (USP 1-May-2019)

Auxiliary Information- Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
KRILL OIL CAPSULES	Natalia Davydova Scientific Liaison +1 (301) 816-8328	NBDS2015 Non-botanical Dietary Supplements 2015

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