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Krill Oil

Change to read:

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.▲2S *(USP41)* 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%▲2S *(USP41)* of astaxanthin.

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, 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▲2S *(USP41)*	13.0
Palmitic acid	16:0	17.0	24.6
Palmitic acid:myristic acid ratio	16:0/14:0	1.6	▲3.6▲2S *(USP41)*
Monounsaturated fatty acids			
Palmitoleic acid	16:1 *n*−7	2.5	▲12.0▲2S *(USP41)*
cis-Vaccenic acid	18:1 *n*−7	4.7	8.0
Oleic acid	18:1 *n*−9	▲6.0▲2S *(USP41)*	14.5

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Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
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▲2S *(USP41)*
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, Line shape standard (^{1}H), Sensitivity standard (^{1}H), Sensitivity standard (^{31}P), 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%.▲2S *(USP41)*

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

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. 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 (^{1}H): 1% chloroform in acetone-d_6

Sensitivity standard (^{1}H): 0.1% ethylbenzene in chloroform-d

Sensitivity standard (^{31}P): 0.0485 M triphenyl phosphate in acetone-d_6

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-d_4) 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, 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 outside 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.

Standard solution: Prepare as directed in the *Sample solution*, using 300–350 mg of USP Krill Oil RS in place of the sample.

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy ⟨761⟩*.)

Magnetic field strength: NLT 300 MHz for ^{1}H frequency

Probe: Direct observe probe capable of tuning to the resonance frequency of ^{31}P (dependent on the specific magnetic field strength used)

Instrument performance qualification

[Note—Testing for sensitivity and line shape should be performed on the interval specified by the manufacturer of the instrument used. Performing these tests on a minimum of a monthly basis is required for this method, but may be done more often, as required. Resolution testing is to be performed during each analysis and documented as a part of the analytical results.]

^{1}H Line shape test: Using the *Line shape standard (^{1}H)* and the protocol recommended by the instrument manufacturer, the instrument must achieve the line shape specifications for the probe in use, as required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 1% chloroform in acetone-d_6 is most commonly used.]

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of the instrument; 0.1% ethylbenzene in chloroform-d is most commonly used.]

^{31}**P Sensitivity test:** Using the *Sensitivity standard* (*^{31}P*) and the protocol recommended by the instrument manufacturer, the instrument must achieve the sensitivity specifications required by the instrument manufacturer. [Note—A different standard solution may be required or recommended by the manufacturer of the instrument; 0.0485 M triphenyl phosphate in acetone-d is most commonly used.]

^{1}**H Resolution test:** The resolution is demonstrated by the ability to detect both of the ^{29}Si satellite signals of TMS. The satellites must be resolved from the TMS signal in the spectrum with a line broadening factor of NMT 0.5 ppm.

^{31}**P Resolution test:** The resolution is demonstrated using the phosphatidylcholine ether peak and the phosphatidylcholine peak. The separation of these peaks (with an applied line broadening factor of 1.0) must be demonstrated, as follows. Using the baseline as a reference, determine the total peak height of the phosphatidylcholine ether peak , and draw a line at 30% of that total peak height (intensity). [Note—The phosphatidylcholine ether signal appears just downfield from the phosphatidylcholine signal.] The phosphatidylcholine ether peak and the neighboring phosphatidylcholine peak must be fully resolved at a point that is NMT 30% of the peak height of the phosphatidylcholine ether peak.

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	^{1}H NMR Qualitative Measurement
Pulse program	^{1}H-decoupled ^{31}P (inverse gated)	Single pulse ^{1}H
Spectral width	50 ppm (25 ppm to −25 ppm)	20 ppm (−3 ppm to 17 ppm)
Transmitter offset	Center of spectral width, 0 ppm	Center of spectral width, 7 ppm
Relaxation delay	5–15 s	2–5 s
Acquisition time	1–6 s	1–6 s
Size of data set	NLT 64k (32k with zero-filling)	NLT 64k (32k with zero-filling)

[Note—The acquisition time is dependent upon the dwell time and the number of data points collected. The number of scans acquired using a 300 MHz instrument must be NLT 512.]

System suitability: Under the conditions outlined in *Data collection*, the ^{31}P NMR signal of triphenyl phosphate should be observed at −17.80 ppm, and the ^{1}H NMR spectrum should be referenced to the ^{1}H signal of TMS (0 ppm) for all spectra acquired in the *Analysis*. For quantitative analysis, a sufficient number of scans should be acquired such that the signal-to-noise ratio for the phosphatidylcholine signal in the ^{31}P spectrum of the *Sample solution* acquired in the *Analysis* is NLT 2000.

Analysis: Acquire the data outlined in *Data collection*. Minimally acquire the ^{1}H spectrum (fingerprint) of the *Sample solution* and the *Standard solution* as well as the quantitative ^{31}P spectrum of the *Sample solution* and the *Standard solution*. Record the resulting spectra, and perform integration by hand or automated means on the quantitative ^{31}P NMR spectrum of the *Sample solution*. Integration of the peaks contained in the spectrum of the *Sample solution* must be performed such that the complete set of phospholipid peaks (as identified by comparison to the spectrum of the *Standard solution* and its reference spectrum) is included in the integration. The integration region for each signal must extend ±0.05 ppm on either side of the ^{31}P signal. Quantify the total phospholipids present, the phosphatidylcholine ether content, and the phosphatidylcholine content in the *Sample solution* using comparison to the concentration of the *Internal standard*. Compare the ^{1}H spectrum of the *Sample solution* to that of the *Standard solution* to determine the similarity of fingerprints according to which phospholipids identified in the reference spectrum of the *Standard solution* are present in the spectrum of the *Sample solution*.

Calculations: Use the following equations and molecular weights listed in *Table 3* to determine the phospholipids content in the sample taken:

$$\mathrm{mmol}_{IS} = (W_{IS} \times C_{IS})/(MW_{IS} \times 100)$$

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)

$$\mathrm{mmol}_{PL} = (I_{PL} \times A_{IS} \times \mathrm{mmol}_{IS})/(I_{IS} \times A_{PL})$$

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)

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

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

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$$C_{PL} = (MW_{PL} \times mmol_{PL} \times 100)/W_S$$

C_{PL} = concentration of the phospholipid of interest in the *Sample solution* (%, w/w)

MW_{PL} = molecular weight of the phospholipid of interest (g/mol, from *Table 3*) in the *Sample solution* (mg)

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

W_S = weight of the sample present in the *Sample solution* (mg)

[Note—Use the molecular weight specified in *Table 3* for the calculations.]

Table 3

Component	Approximate Chemical Shift (ppm) in Reference to Triphenyl Phosphate	Molecular Weight (g/mol)
Triphenyl phosphate (*Internal standard*)	−17.8	—
Phosphatidylcholine (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-lysophosphatidylcholine and 2-lysophosphatidylcholine will depend upon the applied magnetic field strength of the NMR spectrometer used for the test procedure.

Acceptance criteria

Total phospholipids: 30%–59% (w/w)

▲**Sum of phosphatidylcholine, 1-lysophosphatidylcholine, and 2-lysophosphatidylcholine:**▲2S *(USP41)* 60%–96% (w/w) of the *Total phospholipids* content

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. [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:

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

A = absorbance of the *Sample solution*

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

C = concentration of the *Sample solution* (g/mL)

Acceptance criteria: NLT ▲0.005%▲2S *(USP41)*

CONTAMINANTS

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

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by Method No. 1613 Revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by Method No. 1668 Revision A of the Environmental Protection Agency.

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 (polychlorinated biphenyls, non-ortho 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 3.0 pg/g of WHO toxic equivalents.

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• **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: 250 mg/mL of Krill Oil 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, light-resistant containers, and store at controlled room temperature. It may be bottled or otherwise packaged in containers from which air has been expelled by production of a vacuum or by an inert gas.

• **Labeling:** The label states the average content of DHA and EPA in mg/g. It also states the name and concentration of any added antioxidant.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Astaxanthin Esters from *Haematococcus pluvialis* RS

USP Astaxanthin (Synthetic) RS

▲▲ (CN 1-May-2018)

USP Krill Oil RS

▲▲ (CN 1-May-2018)

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

Topic/Question	Contact	Expert Committee
KRILL OIL	Fatkhulla K Tadjimukhamedov Associate Scientific Liaison +1 (301) 230-3216	NBDS2015 Non-botanical Dietary Supplements 2015

Chromatographic Columns Information: Chromatographic Columns

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