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Crypthecodinium cohnii Oil

DEFINITION
Crypthecodinium cohnii Oil is obtained from the fermentation and extraction of algae of the species *Crypthecodinium cohnii* and contains NLT 35.0% (w/w) of docosahexaenoic acid (DHA, C₂₂H₃₂O₂) (C22: 6 n-3), as the only significant polyunsaturated fatty acid present. Suitable antioxidants in appropriate concentration may be added.

IDENTIFICATION
• **A. LONG-CHAIN UNSATURATED FATTY ACID PROFILE:** Proceed as directed in [Fats and Fixed Oils \(401\)](#), [Omega-3 Fatty Acids Determination and Profile](#), [Content of EPA and DHA](#).

Analysis
Samples: *Standard Solution 2a*, *Standard Solution 2b*, and *Test Solution 1*
Calculate the area percentage for each fatty acid as methyl ester in *Test Solution 1*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual fatty acid as methyl ester
 r_T = sum of all the peak responses, except the solvent and butylated hydroxytoluene peaks

Acceptance criteria: The retention times of the peaks for the docosahexaenoic acid methyl ester and the eicosapentaenoic acid methyl ester from *Test Solution 1* correspond to those from *Standard Solution 2a* and *Standard Solution 2b*, respectively. The area percentage for the methyl esters of the fatty acids from *Test Solution 1* meets the requirements for each fatty acid indicated in [Table 1](#).

Table 1

Fatty Acid	Relative Retention Time	Shorthand Notation	Lower Limit (Area, %)	Upper Limit (Area, %)
Linoleic acid	0.52	18:2 n-6	0	1.0
Eicosapentaenoic acid	0.79	20:5 n-3	0	0.1
Docosapentaenoic acid	0.94	22:5 n-6	0	0.1
Docosahexaenoic acid	1.00	22:6 n-3	35.0	47.0

COMPOSITION
• **CONTENT OF DHA**
Analysis: Proceed as directed in [Fats and Fixed Oils \(401\)](#), [Omega-3 Fatty Acids Determination and Profile](#), [Content of EPA and DHA](#).
Acceptance criteria: NLT 35.0% (w/w) of docosahexaenoic acid (DHA)

IMPURITIES
Change to read:
• **LIMIT OF ARSENIC**
[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytef, and plastic vessels before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of arsenic as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytef, and plastic vessels before use by soaking in warm 8 N [nitric acid](#) for 30 min and by rinsing with deionized water.]



Solution A: Transfer 1 g of ultrapure palladium metal to a Teflon beaker. Add 20 mL of [water](#) and 10 mL of [nitric acid](#), and warm on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of [water](#) and 1 mL of [nitric acid](#), and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.

Solution C: *Solution A*, *Solution B*, and 2% [nitric acid](#) (3:2:5). A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed in ▲[Arsenic \(211\), Procedures, Procedure 1 and Procedure 2](#)▲ (CN 1-Jun-2023), to a 100-mL volumetric flask. Add 40 mL of [water](#) and 5 mL of [nitric acid](#), and dilute with [water](#) to volume. This solution contains 0.10 µg/mL of arsenic.

Blank: [Nitric acid](#) and [water](#) (1:19)

Standard solutions: Dilute the *Standard stock solution* with the *Blank* to obtain concentrations of 0.002, 0.005, 0.010, 0.025, and 0.050 µg/mL of arsenic.

Sample solution: For preparation, use a microwave oven with a magnetron frequency of 2455 MHz and a selectable output power of 0–950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytetrafluoroethylene liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes. [CAUTION—Wear proper eye protection and protective clothing and gloves.] Transfer approximately 500 mg of *Cryptocodinium cohnii* Oil, weighed to the nearest 0.1 mg, into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of [nitric acid](#), and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min, followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature. [NOTE—A cool water bath may be used to speed the cooling process.] Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven, and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with [water](#) to volume.

Analysis: Program the graphite furnace as follows. Dry at 115°, using a 1-s ramp, a 65-s hold, and an argon flow of 300 mL/min; char the sample at 1000°, using a 1-s ramp, a 20-s hold, and an airflow of 300 mL/min; cool down, and purge the air from the furnace for 10 s, using a 20° set temperature and an argon flow of 300 mL/min; atomize at 2400°, using a 0-s ramp and a 5-s hold with the argon flow stopped; and clean out at 2600° with a 1-s ramp and a 5-s hold. Separately inject equal volumes (20 µL) of the *Standard solutions*, the *Sample solution*, and the *Blank*, followed by an injection of 5 µL of *Solution C* for each of the samples, into the graphite tube of a suitable graphite furnace atomic absorption spectrophotometer equipped with a hollow-cathode lamp for arsenic. Determine the peak area at the arsenic emission line at 193.7 nm, corrected for background absorption. Plot the corrected peak areas of the *Standard solutions* versus their contents of arsenic, in µg/mL, and calculate the regression line best fitting the points. Determine the concentration, *C*, in µg/mL, of arsenic in each mL of the *Sample solution* by interpolation from the regression line.

Calculate the content of arsenic in the portion of *Cryptocodinium cohnii* Oil taken:

$$\text{Result} = (C/W) \times 25$$

C = concentration, as obtained above

W = weight of *Cryptocodinium cohnii* Oil taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glass, polytetrafluoroethylene, and plastic vessels before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glass, polytetrafluoroethylene, and plastic vessels before use by soaking in warm 8 N [nitric acid](#) for 30 min and by rinsing with deionized water.]

Solution A: 10 g of ultrapure monobasic ammonium phosphate in 1 mL of [nitric acid](#) and 40 mL of [water](#) to dissolve the phosphate. Dilute with deionized water to 100 mL.

Solution B: Transfer 1 g of ultrapure magnesium nitrate to a Teflon beaker. Add 40 mL of [water](#) and 1 mL of [nitric acid](#), and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it to a 100-mL volumetric flask, and dilute with deionized water to volume.