Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 15-cm; 5-µm packing L79[1]
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate are about 1 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.5 between L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate
Analysis
Sample: *Sample solution*
Calculate the percentage of D-5-methyltetrahydrofolate in the portion of calcium L-5-methyltetrahydrofolate taken:

$$\text{Result} = [r_D/(r_D + r_L)] \times 100$$

r_D = peak response of D-5-methyltetrahydrofolate from the *Sample solution*
r_L = peak response of L-5-methyltetrahydrofolate from the *Sample solution*
Acceptance criteria: NMT 1.0%

PERFORMANCE TESTS
- **DISINTEGRATION AND DISSOLUTION ⟨2040⟩:** Meet the requirements for *Disintegration*
- **WEIGHT VARIATION ⟨2091⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store in a tight, light-resistant container, in a cool and dry place.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Calcium D,L-5-Methyltetrahydrofolate RS
  USP Folic Acid RS■1S (USP37)

Chondroitin Sulfate Sodium

Chondroitin, hydrogen sulfate, sodium salt [9082-07-9].

DEFINITION
Chondroitin Sulfate Sodium is the sodium salt of the sulfated linear glycosaminoglycan obtained from bovine, porcine, or avian cartilages of healthy and domestic animals used for food by humans. Chondroitin Sulfate Sodium consists mostly of the sodium salt of the sulfate ester of *N*-acetylchondrosamine (2-acetamido-2-deoxy-β-D-galactopyranose) and D-glucuronic acid copolymer. These hexoses are alternately linked β-1,4 and β-1,3 in the polymer. Chondrosamine moieties in the prevalent glycosaminoglycan are monosulfated primarily on position 4 and less so on position 6. It contains NLT 90.0% and NMT 105.0% of chondroitin sulfate sodium, calculated on the dried basis.
[NOTE—Chondroitin Sulfate Sodium is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]

[1] A chiral-recognition protein, human serum albumin (HSA), chemically bonded to silica particle, about 5 µm in diameter. For example: Chromtech Chiral HSA, available at www.chromtech.com.

IDENTIFICATION
- **A. INFRARED ABSORPTION ⟨197K⟩**
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* **⟨191⟩:** Meets the requirements
  Sample solution: 0.5 g in 10 mL of water

Add the following:

■• **C. DISACCHARIDE COMPOSITION**
The chromatogram of the enzymatically digested *Sample solution* as obtained in the test for *Limit of Nonspecific Disaccharides* shows three main peaks corresponding to dehydrated glucuronic acid-[1→3]-chondrosamine-4-sulfated (ΔDi-4S), dehydrated glucuronic acid-[1→3]-chondrosamine-6-sulfated (ΔDi-6S), and nonsulfated dehydrated glucuronic acid-[1→3]-chondrosamine (ΔDi-0S) in the enzymatically digested *Standard solution*. By peak-area response, ΔDi-4S is the most abundant, followed by ΔDi-6S, with ΔDi-0S being the least abundant of the three. The ratio of the peak response of the ΔDi-4S to the ΔDi-6S is NLT 1.0.■1S (USP37)

Add the following:

■• **D. SPECIFIC ROTATION:** Meets the requirements for *Optical Rotation, Specific Rotation* ⟨781S⟩ in *Specific Tests*■1S (USP37)

COMPOSITION
- **CONTENT OF CHONDROITIN SULFATE SODIUM**
  Standard solutions: 1.5, 1.0, and 0.5 mg/mL of USP Chondroitin Sulfate Sodium RS in water
  Sample solution: Transfer 100 mg of dried Chondroitin Sulfate Sodium into a 100-mL volumetric flask, dissolve in 30 mL of water, and dilute with water to volume.
  Diluent: Weigh about 297 mg of monobasic potassium phosphate, 492 mg of dibasic potassium phosphate, and 250 mg of polysorbate 80, and transfer into a 1-L beaker. Dissolve in 900 mL of water, and adjust with potassium hydroxide or phosphoric acid to a pH of 7.0 ± 0.2. Dilute with water to 1 L, and mix thoroughly.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Photometric titration
  Titrant: 1 mg/mL of cetylpyridinium chloride in water. Degas before use.
  Endpoint detection: Turbidimetric with a photoelectric probe
  Analysis: Transfer 5.0 mL each of the *Standard solution* and the *Sample solution* to separate titration vessels, and add 25 mL of *Diluent* to each. Stir until a steady reading is obtained with a phototrode either at 420, 550, or 660 nm. Set the instrument to zero in absorbance mode. Titrate with *Titrant* using the phototrode to determine the endpoint turbidimetrically. From a linear regression equation, calculated using the volumes of *Titrant* consumed versus concentrations of the *Standard solutions*, determine the concentration of chondroitin sulfate sodium in the *Sample solution*.
  Calculate the percentage of chondroitin sulfate sodium in the portion of Chondroitin Sulfate Sodium taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of chondroitin sulfate sodium in the aliquot of the *Sample solution*, obtained from the regression equation (mg/mL)
C_U = concentration of Chondroitin Sulfate Sodium in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–105.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION ⟨281⟩:** 20.0%–30.0% on the dried basis

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- **Chloride and Sulfate,** *Chloride* ⟨221⟩**:** NMT 0.50%; a 0.10-g portion shows no more chloride than corresponds to 0.7 mL of 0.020 N hydrochloric acid.
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩
  Sample solution: Dissolve 200 mg in 40 mL of water. Add 10 mL of a 30-mg/mL solution of cetylpyridinium chloride, pass through a filter, and use a 25-mL portion of the filtrate.
  Acceptance criteria: NMT 0.24%; the *Sample solution* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid.

Change to read:

- **Electrophoretic Purity**
  ■[**Caution—**Voltages used in electrophoresis can readily deliver a lethal shock. The hazard is increased by the use of aqueous buffer solutions and the possibility of working in damp environments. The equipment, with the possible exception of the power supply, should be enclosed in either a grounded metal case or a case made of insulating material. The case should have an interlock that deenergizes the power supply when the case is opened, after which reactivation should be prevented until activation of a reset switch is carried out. High-voltage cables from the power supply to the apparatus should preferably be a type in which a braided metal shield completely encloses the insulated central conductor, and the shield should be grounded. The base of the apparatus should be grounded metal or contain a grounded metal rim which is constructed in such a way that any leakage of electrolyte will produce a short which will deenergize the power supply before the electrolyte can flow beyond the protective enclosure. If the power supply contains capacitors as part of a filter circuit, it should also contain a bleeder resistor to ensure discharge of the capacitors before the protective case is opened. A shorting bar that is activated by opening the case may be considered as an added precaution. Because of the potential hazard associated with electrophoresis, laboratory personnel should be completely familiar with electrophoresis equipment before using it.]■1S (*USP37*)
  Barium acetate buffer: Dissolve 25.24 g of barium acetate in 900 mL of water. Adjust with acetic acid to a pH of 5.0, and dilute with water to 1000 mL.
  Staining reagent: Dissolve 1 g of toluidine blue in 1000 mL of 0.1 M acetic acid.
  Standard solution A: 30 mg/mL of USP Chondroitin Sulfate Sodium RS in water
  Standard solution B: Dilute 1 mL of *Standard solution A* with water to 50 mL.
  Sample solution: 30 mg/mL of Chondroitin Sulfate Sodium in water
  Analysis: Fill the chambers of an electrophoresis apparatus suitable for separations on cellulose acetate membranes[1] (a small submarine gel chamber or one dedicated to membrane media) with *Barium acetate buffer*. Soak a cellulose acetate membrane, 5–6 cm × 12–14 cm, in *Barium acetate buffer* for 10 min, or until evenly wetted, then blot dry between two sheets of absorbent paper. Using an applicator[2] suitable for electrophoresis, apply equal volumes (0.5 µL) of the *Sample solution, Standard solution A,* and *Standard solution B* to the brighter side of the membrane held in position in an appropriate applicator stand or on a separating bridge in the chamber. Ensure that both ends of the membrane are dipped at least 0.5–1.0 cm deep into the buffer chambers. Apply a constant 60 volts (6 mA at the start) for 2 h. [Note—Perform the application of solutions and voltage within 5 min because further drying of the blotted paper reduces sensitivity.]
  Place the membrane in a plastic staining tray, and with the application side down, float or gently immerse in *Staining reagent* for 5 min. Then stir the solution gently for 1 min. Remove the membrane, and destain in 5% acetic acid until the background clears. Compare the bands. [Note—Document the results by taking a picture within 15 min of completion of destaining.]
  Acceptance criteria: The electropherogram from the *Sample solution* exhibits a major band that is identical in position to the band from *Standard solution A*. The band from *Standard solution B* is clearly visible at a mobility similar to the band from *Standard solution A*. Any secondary band in the electropherogram of the *Sample solution* is not more intense than the band from *Standard solution B*. NMT 2% of any individual impurity is found. [Note—Document the results by taking a picture within 15 min of completion of destaining.]
- **Limit of Protein**
  Solution A: 20 mg/mL of sodium tartrate dihydrate
  Solution B: 10 mg/mL of cupric sulfate
  Solution C: 20 mg/mL of anhydrous sodium carbonate in 0.1 M sodium hydroxide
  Dilute Folin-Ciocalteu reagent: Dilute Folin-Ciocalteu phenol TS with water (1:5). Prepare immediately before use.
  Alkaline cupric tartaric reagent: Mix 1 mL each of *Solution A* and *Solution B*, and to the mixture slowly add 100 mL of *Solution C* with stirring. Use within 24 h, and discard afterward.
  Standard solution: 36 µg/mL of bovine serum albumin certified standard in water
  Sample solution: Transfer a portion of Chondroitin Sulfate Sodium, equivalent to 60 mg of the dried substance, to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.
  Instrumental conditions
  (See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
  Analytical wavelength: 750 nm
  Blank: Water
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Add 2.0 mL of freshly prepared *Alkaline cupric tartaric reagent* to test tubes containing 2.0 mL of the *Standard solution*, 2.0 mL of the *Sample solution*, or 2.0 mL of the *Blank*. After 10 min, add 1.0 mL of *Dilute Folin-Ciocalteu reagent* to each test tube, and mix immediately and vigorously. After 30 min, measure the absorbance of the *Standard solution* and *Sample solution* against the *Blank*.
  Acceptance criteria: NMT 6.0% on the dried basis; the absorbance of the *Sample solution* is NMT the absorbance of the *Standard solution*.

CONTAMINANTS

- **Microbial Enumeration Tests** ⟨2021⟩**:** The total bacterial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.
- **Absence of Specified Microorganisms** ⟨2022⟩**:** It meets the requirements of the tests for absence of *Salmonella* species, and *Escherichia coli*.
- **Heavy Metals,** *Method II* ⟨231⟩**:** NMT 20 ppm

SPECIFIC TESTS

Add the following:

■• **Limit of Nonspecific Dissaccharides**
  Solution A: Water adjusted with 0.1 N hydrochloric acid to a pH of 3.5

[1] Suitable cellulose acetate membranes for electrophoresis are available from Malta Chemetron SRL, Milano, Italy; Fluka Chemical Corp., Milwaukee, WI; and DiaSys Corp., Waterbury, CT (www.diasys.com).
[2] Suitable applicators are available from DiaSys Corp., Waterbury, CT (www.diasys.com) and Helena Laboratories, Beaumont, TX (www.helena.com).

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Solution B: 1 M sodium chloride adjusted with 0.1 N hydrochloric acid to a pH of 3.5
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.5	100	0
21.0	61	39
21.1	100	0

Buffer solution: 50 mM tris(hydroxymethyl)aminomethane and 60 mM sodium acetate (1:1), adjusted with diluted hydrochloric acid to a pH of 8.0
Chondroitinase AC solution: Combine 2 units of chondroitinase AC[3] and 0.5 mL of *Buffer solution*. Dilute with water to 10.0 mL, and mix thoroughly.
Enzyme suitability: Dilute the digested *Standard solution* (see *Analysis* section below) (1 in 10), and measure the absorbance at 230 nm in 1-cm path cells. Calculate the absorptivity of the USP Chondroitin Sulfate Sodium RS:

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

A = absorbance of the diluted and digested *Standard solution*
C = concentration of USP Chondroitin Sulfate Sodium RS in the *Standard solution* (mg/mL)
D = dilution factor of digested *Standard solution* (1/5)
d = dilution factor for the UV measurement (1/10)

Enzyme suitability requirements: The absorptivity of the digested USP Chondroitin Sulfate Sodium RS is NLT 8 AU · mL · mg^{-1} · cm^{-1}.
Standard solution: 2.4 mg/mL of dried USP Chondroitin Sulfate Sodium RS in water
Sample solution: Transfer about 250 mg of dried (105° for 4 h) Chondroitin Sulfate Sodium to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.
System suitability solution: Add 1 volume of *Standard solution* to 1 volume of *Sample solution*.
Blank: Water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L14
Flow rate: 1 mL/min
Injection volume: 25 µL
[NOTE—The *Injection volume* may be decreased to improve the peak shape of the analytes.]
System suitability
Samples: *Standard solution* and *System suitability solution* (prepared as directed for *Samples* in the *Analysis* below)
[NOTE—The relative retention times for the ΔDi-0S, ΔDi-6S, and ΔDi-4S peaks are 0.80, 0.97, and 1.0, respectively.]
Suitability requirements
Chromatogram similarity: The chromatogram of the *Standard solution* is similar to that of the Reference Chromatogram provided with USP Chondroitin Sulfate Sodium RS.
Resolution: NLT 2.0, between the ΔDi-4S and ΔDi-6S peaks
Recovery factor: NLT 95% of the USP Chondroitin Sulfate Sodium RS added to the *Sample solution*
[NOTE—This test is intended to demonstrate the absence of enzyme inhibition by impurities in the samples. Performance of this test is required only for samples not meeting the *Acceptance criteria* below. The recovery factor can be calculated as follows.

$$\text{Result} = [(2 \times \Sigma r_{SY}) - \Sigma r_U]/\Sigma r_S \times 100$$

Σr_{SY} = sum of the peak areas of ΔDi-0S, ΔDi-4S, and ΔDi-6S from the *System suitability solution*
Σr_U = sum of the peak areas of ΔDi-0S, ΔDi-4S, and ΔDi-6S from the *Sample solution*
Σr_S = sum of the peak areas of ΔDi-0S, ΔDi-4S, and ΔDi-6S from the *Standard solution*]

Relative standard deviation: NMT 5.0% for the ΔDi-0S, ΔDi-4S, and ΔDi-6S peaks
Analysis
Samples: *Standard solution*, *Sample solution*, *Sytem suitability solution*, and *Blank*
In four separate vials, combine 4 volumes of *Chondroitinase AC solution* with 1 volume each of *Standard solution*, *Sample solution*, *System suitability solution*, and *Blank*. Mix thoroughly. Incubate at 37°C for 3 h. Allow to cool before injection.
Calculate the percentage of specific disaccharides in the sample taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times (C_S/C_U) \times 100$$

Σr_U = sum of the peak areas of ΔDi-0S, ΔDi-4S, and ΔDi-6S from the *Sample solution*
Σr_S = sum of the peak areas of ΔDi-0S, ΔDi-4S, and ΔDi-6S from the *Standard solution*
C_S = concentration of chondroitin sulfate sodium in the *Standard solution* (mg/mL)
C_U = concentration of Chondroitin Sulfate Sodium in the *Sample solution* (mg/mL)

Calculate the content of nonspecific disaccharides in the sample taken:

$$\text{Result} = CSC - SDC$$

CSC = Chondroitin sulfate sodium content from the test for *Content of Chondroitin Sulfate Sodium* (%)
SDC = specific disaccharides content (%)

Acceptance criteria: NMT 10.0%■1S (USP37)

- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: Transfer 2.5 g of Chondroitin Sulfate Sodium to a 50-mL volumetric flask. Dissolve in and dilute with carbon dioxide-free water to volume, and examine immediately.
  Instrumental conditions
  (See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
  Analytical wavelength: 420 nm
  Cell: 1 cm
  Blank: Carbon dioxide-free water
  Analysis: Measure the absorbance of the *Sample solution*.
  Acceptance criteria: Its absorbance is NMT 0.35.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** –20.0° to –30.0°
  Sample solution: 30 mg/mL
- **pH** ⟨**791**⟩**:** 5.5–7.5, in a solution (1 in 100)
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 4 h: it loses NMT 12.0% of its weight. [NOTE—Chondroitin Sulfate Sodium is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]

■[3] Chondroitinase AC from Chromadex, part number ASB-00003613-10.
■1S (USP37)

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ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to state the source(s) from which the article was derived, whether bovine, porcine, avian, or a mixture of any of them.
- **USP Reference Standards** ⟨11⟩
  USP Chondroitin Sulfate Sodium RS

Fish Oil Containing Omega-3 Acids

DEFINITION

Fish Oil Containing Omega-3 Acids is the purified, winterized, and deodorized fatty oil obtained from fish of the families Engraulidae, Carangidae, Clupeidae, Osmeridae, Scombroidae, and Ammodytidae. The omega-3 acids are defined as the following: alpha-linolenic acid (C18:3 n–3), moroctic acid (C18:4 n–3), eicosatetraenoic acid (C20:4 n–3), eicosapentaenoic acid (EPA) (C20:5 n–3), heneicosapentaenoic acid (C21:5 n–3), docosapentaenoic acid (C22:5 n–3), and docosahexaenoic acid (DHA) (C22:6 n–3). It contains NLT 28.0% (w/w) of total omega-3 acids, expressed as free acids, consisting of NLT 13.0% of EPA and NLT 9.0% of DHA. Suitable antioxidants in appropriate concentrations may be added.

IDENTIFICATION

Change to read:

- **A.** ■The retention times of the docosahexaenoic acid methyl ester and eicosapentanoic acid methyl ester peaks from *Test solution 1* in *Content of EPA and DHA* correspond to those of the docosahexaenoic acid methyl ester and eicosapentanoic acid methyl ester peaks from *Standard solution 2a* and *Standard solution 2b*, respectively, in *Fats and Fixed Oils* ⟨401⟩, *Content of EPA and DHA*.■1S (USP37) The sum of the area for EPA and DHA methyl esters is NLT 22% of the total detected area for the methyl esters, and no other peak has an area higher than 20% of the total detected area for the methyl esters. ■In addition to the EPA and DHA peaks, *Test solution 1* exhibits at least 15 more peaks with retention times similar to those of the *Fish oil standard solution*, as obtained in the test for *Content of EPA and DHA*.■1S (USP37)

COMPOSITION

Change to read:

- **Content of EPA and DHA**
  (See *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.)
  ■**Standard solution 1a, Standard solution 1b, Standard solution 2a, Standard solution 2b, Test solution 1, Test solution 2, System suitability solution 1, System suitability solution 2, Chromatographic system, System suitability**, and **Analysis:** Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Content of EPA and DHA* for triglycerides.
  Fish oil standard solution: Transfer 300 mg of USP Fish Oil RS into a 10-mL volumetric flask, and dissolve in and dilute with *Antioxidant Solution* to volume. Proceed as directed for *Test Solution 1 (for triglycerides)* in *Fats and Fixed Oils* ⟨401⟩, *Content of EPA and DHA*, starting with "Transfer 2.0 mL".
  Identify the relevant fatty acid methyl esters in the *Fish oil standard solution* by comparing their retention times with those in the reference chromatogram supplied with the USP Fish Oil RS.■1S (USP37)
  Acceptance criteria: NLT 13.0% (w/w) of EPA and NLT 9.0% (w/w) of DHA

Change to read:

- **Content of Total Omega-3 Acids**
  (See *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.)
  Analysis: ■Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Content of Total Omega-3-Acids* (for triglycerides).■1S (USP37)
  Acceptance criteria: NLT 28.0% (w/w) of total omega-3 acids, expressed as free acids

CONTAMINANTS
- **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 first through a strong-acid, strong-base, mixed-bed ion-exchange resin. 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.]
  1% Palladium stock solution: Transfer 1 g of ultrapure palladium metal into 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 into a 100-mL volumetric flask, and dilute with deionized water to volume.
  1% Magnesium nitrate stock solution: Transfer 1 g of ultrapure magnesium nitrate into 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 into a 100-mL volumetric flask, and dilute with deionized water to volume.
  Modifier working solution: *1% Palladium stock solution*, *1% Magnesium nitrate stock solution*, 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.
  Blank: Nitric acid and water (5 in 100)
  Standard stock solution: Transfer 10.0 mL of *Standard Arsenic Solution*, prepared as directed in *Arsenic* ⟨211⟩, 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.
  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 of the *Sample solution*, 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 polytef 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 Fish Oil Containing Omega-3 Acids, 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

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