Chondroitin Sulfate Sodium, Shark

Type of Posting	Revision Bulletin
Posting Date	27–Jan–2017
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Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has revised the Chondroitin Sulfate Sodium, Shark monograph. The purpose for the revision is to lower the ΔDi-2,6diS limit from NLT 15% to NLT 8% the accommodate products on the market.

Minor editorial changes have been made to update the monograph to the current *USP* style.

The Chondroitin Sulfate Sodium, Shark Revision Bulletin supersedes the currently official Chondroitin Sulfate Sodium, Shark monograph. The Revision Bulletin will be incorporated in the *Second Supplement* to *USP 40–NF 35*.

Should you have any questions, please contact Huy Dinh, Senior Scientific Liaison (301-816-8594 or htd@usp.org).

Chondroitin Sulfate Sodium, Shark

Chondroitin, hydrogen sulfate, sodium salt [9007-28-7].

DEFINITION

Change to read:

Chondroitin Sulfate Sodium, Shark is the sodium salt of the sulfated linear glycosaminoglycan obtained from shark cartilages used for human foods. Chondroitin Sulfate Sodium, Shark 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 6 and less so on position 4 with minor disulfation on both positions 4 and 6. NLT •8%• (RB 1-Feb-2017) of the D-glucuronic acid moieties are monosulfated on position 2. It contains NLT 90.0% and NMT 105.0% of chondroitin sulfate sodium, calculated on the dried basis.

[NOTE—Chondroitin Sulfate Sodium, •Shark• (RB 1-Feb-2017) is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]

IDENTIFICATION

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

Change to read:

- **C. SPECIFIC DISACCHARIDES:** The chromatogram of the enzymatically digested *Sample solution* as obtained in the test for *Disaccharide Composition* shows three main peaks due to 6-sulfated (ΔDi-6S), 4-sulfated (ΔDi-4S), and 2,6-disulfated (ΔDi-2,6diS) disaccharides, corresponding to those of the enzymatically digested *Standard solution*, with ΔDi-6S being the most abundant, followed by ΔDi-4S, with NLT •8%• (RB 1-Feb-2017) corresponding to ΔDi-2,6diS. Additional minor peaks corresponding to non-sulfated (ΔDi-0S) and 4,6 disulfation may be detected.
- **D. SPECIFIC ROTATION:** Meets the requirements in the *Specific Tests*

COMPOSITION

- **CONTENT OF CHONDROITIN SULFATE SODIUM**
  Standard solutions: 1.5, 1.0, and 0.5 mg/mL of dried USP Chondroitin Sulfate Sodium, Shark RS in water
  Sample solution: Transfer 100 mg of dried Chondroitin Sulfate Sodium, Shark 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 to 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 the photoelectric probe set either at 420, 550, or 660 nm. Set the instrument to zero in absorbance mode. Titrate with *Titrant* using the photoelectric probe 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, Shark 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, Shark in the *Sample solution* (mg/mL)
  Acceptance criteria: 90.0%–105.0% on the dried basis

Change to read:

- **DISACCHARIDE COMPOSITION**
  Solution A: Water adjusted with 0.1 N hydrochloric acid to a pH of 3.5
  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
4.0	100	0
45.0	50	50
45.1	100	0

  Buffer solution: 50 mM tris(hydroxymethyl)aminomethane and 60 mM sodium acetate, adjusted with diluted hydrochloric acid to a pH of 8.0
  Standard solution: 2.4 mg/mL of dried USP Chondroitin Sulfate Sodium, Shark RS in water
  Sample solution: Transfer about 250 mg of dried Chondroitin Sulfate Sodium, Shark to a 100-mL volumetric flask, and dissolve and dilute with water to volume. Filter to obtain a clear solution.
  Blank: Water
  Chondroitinase ABC solution: Dissolve 1 unit (U/mg of protein) of chondroitinase ABC[1] in •1.0 mL of *Buffer solution*.• (ERR 1-Jun-2016) Mix thoroughly.
  Chondroitinase ABC solution suitability: Dilute the incubated *Standard solution* (1 in 10), and measure its absorbance against the incubated *Blank* at 232 nm. The absorptivity is NLT 8 AU · mL · mg^{-1} · cm^{-1}.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)

[1] Chondroitinase ABC from *Proteus vulgaris* is available from Sigma (www.sigmaaldrich.com), Catalog Number C3667.

Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 25-cm; 5-µm packing L14
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution* (prepared per *Analysis* below)
[NOTE—The relative retention times for the Δ Di-0S, Δ Di-6S, Δ Di-4S, and Δ Di-2,6diS peaks are 0.50, 0.75, 0.80, and 1.0, respectively.]
Suitability requirements
Chromatogram similarity: The chromatogram of the *Standard solution* is similar to the reference chromatogram provided with USP Chondroitin Sulfate Sodium, Shark RS.
Resolution: NLT 2.0 between the Δ Di-6S and Δ Di-4S peaks
Relative standard deviation: NMT 5.0% for the Δ Di-6S, Δ Di-4S, or Δ Di-2,6diS peaks
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
In three separate vials, combine 0.8 mL of *Buffer solution*, 0.1 mL of *Chondroitinase ABC solution*, ● and 0.1 mL ● (ERR 1-Jun-2016) each of the *Standard solution*, *Sample solution*, and *Blank*. Mix thoroughly. Incubate at 37° for 3 h. Allow the solution to cool to room temperature, and centrifuge prior to injection.
Calculate the percentage of each disaccharide in the sample taken:

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

r_U = peak area of Δ Di-0S, Δ Di-6S, Δ Di-4S, or Δ Di-2,6diS from the *Sample solution*
Σr_U = sum of the peak areas of Δ Di-0S, Δ Di-6S, Δ Di-4S, and Δ Di-2,6diS from the *Sample solution*
Acceptance criteria: The area percentage of the Δ Di-6S peak is greater than that of the Δ Di-4S peak, and the area percentage of the Δ Di-2,6diS peak is the lowest of the three. The area percentage of the Δ Di-2,6diS peak is NLT ● 8%. ● (RB 1-Feb-2017)

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** 20.0%–30.0% on the dried basis
- **CHLORIDE AND SULFATE ⟨221⟩,** *Chloride*
  Standard solution: 0.7 mL of 0.020 N hydrochloric acid
  Sample: 0.1 g
  Acceptance criteria: NMT 0.50%
- **CHLORIDE AND SULFATE ⟨221⟩,** *Sulfate*
  Standard solution: 0.25 mL of 0.020 N sulfuric acid
  Sample solution: Dissolve 200 mg in 40 mL of water. Add 10 mL of a solution of cetylpyridinium chloride having a concentration of 30 mg/mL, and pass through a filter. Use a 25-mL portion of the filtrate.
  Acceptance criteria: NMT 0.24%; the *Sample solution* shows no more sulfate than that of the *Standard solution*.
- **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.]
  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, Shark 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, Shark in water
  Analysis: Fill the chambers of an electrophoresis apparatus suitable for separations on cellulose acetate membranes[2] (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[3] 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 the *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 the 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 in Chondroitin Sulfate Sodium, Shark is found.

[2] Suitable cellulose acetate membranes for electrophoresis are available from Malta Chemetron SRL, Milano, Italy; Fluka Chemical Corp., Milwaukee, WI; and Apacor Ltd., Berkshire, England (www.apacor.com/products/electrophoresis/cellulose-acetate-membranes).
[3] Suitable applicators are available from Apacor Ltd., Berkshire, England (www.apacor.com/PDF/APA092-ElectrophoresisEquipmentSupplies.pdf) and Helena Laboratories, Beaumont, TX (www.helena.com).

Change to read:

- **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, Shark, 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 •*Ultraviolet-Visible Spectroscopy* ⟨857⟩.)• (ERR 1-Jun-2016)
  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 three test tubes, each 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

- **ELEMENTAL IMPURITIES—PROCEDURES** ⟨**233**⟩
  Acceptance criteria
  Arsenic: NMT 2.0 µg/g
  Cadmium: NMT 1.0 µg/g
  Lead: NMT 1.0 µg/g
  Mercury: NMT 1.0 µg/g
- **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*.

SPECIFIC TESTS

Change to read:

- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: Transfer 2.5 g of Chondroitin Sulfate Sodium, Shark to a 50-mL volumetric flask. Dissolve in and dilute with carbon dioxide-free water to volume, and examine immediately.
  Instrumental conditions
  (See •*Ultraviolet-Visible Spectroscopy* ⟨857⟩.)• (ERR 1-Jun-2016)
  Analytical wavelength: 420 nm
  Cell: 1 cm
  Blank: Carbon dioxide-free water
  Analysis: Measure the absorbance of the *Sample solution*.
  Acceptance criteria: NMT 0.35
- **OPTICAL ROTATION** ⟨**781S**⟩**,** *Specific Rotation*
  Sample solution: 30 mg/mL in water
  Acceptance criteria: –12.0° to –23.0°
- **pH** ⟨**791**⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: 5.5–7.5
- **LOSS ON DRYING** ⟨**731**⟩
  Analysis: Dry a sample at 105° for 4 h. [NOTE—Chondroitin Sulfate Sodium, Shark is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]
  Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to state the source(s) from which the article was derived.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Chondroitin Sulfate Sodium, Shark RS