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 $\Delta$Di-2,6diS limit from NLT 15% to NLT 8% to 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-acetylcylchondrosamine (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. Chondrosamines 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 98% of the D-glucuronic acid moieties are monosulfated on position 2. It contains NLT 90.0% and NMT 105.0% from the regression equation (mg/mL) of chondroitin sulfate sodium, calculated on the dried basis.

[NOTE—Chondroitin Sulfate Sodium, Shark 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 nonsulfated (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 RS 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:

\[
\text{Result} = \left( \frac{C}{C_0} \right) \times 100
\]

\[C = \text{concentration of chondroitin sulfate sodium in the aliquot of the Sample solution, obtained from the regression equation (mg/mL)}\]

\[C_0 = \text{concentration of Chondroitin Sulfate Sodium, Shark in the Sample solution (mg/mL)}\]

**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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>45.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>45.1</td>
<td>100</td>
<td>0</td>
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</tbody>
</table>

**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 in 1.0 mL of Buffer solution. 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 absorboptivity 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.
Chondroitin

Mode: LC
Detector: UV 232 nm
Column: 4.6-mm x 25-cm; 5-µm packing L14
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: Standard solution (prepared per Analysis below)

Acceptance criteria: NMT 0.50% gently for 1 min. Remove the membrane, and destain Sample:

Sample solution: Dissolve 200 mg in 40 mL of water. [0.25 mL of 0.020 N sulfuric acid pare the bands. Standard solution:

Standard solution: 0.7 mL of 0.020 N hydrochloric Place the membrane in a plastic staining tray, and with the application side down, float or gently immerse in

Acceptance criteria: NMT 5.0% for the 

 ΔDi-6S, ΔDi-4S, or ΔDi-2,6diS peaks

Relative standard deviation: NMT 5.0% for the 

 ΔDi-6S, ΔDi-4S, or ΔDi-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 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:

Result = \( \frac{r_U}{\Sigma r_U} \times 100 \)

\( r_U \) = peak area of \( \Delta \text{Di-0S}, \Delta \text{Di-6S}, \Delta \text{Di-4S}, \text{ or } \Delta \text{Di-2,6diS} \) from the Sample solution

\( \Sigma r_U \) = sum of the peak areas of \( \Delta \text{Di-0S}, \Delta \text{Di-6S}, \Delta \text{Di-4S}, \text{ and } \Delta \text{Di-2,6diS} \) from the Sample solution

Acceptance criteria: The area percentage of the \( \Delta \text{Di-6S} \) peak is greater than that of the \( \Delta \text{Di-4S} \) peak, and the area percentage of the \( \Delta \text{Di-2,6diS} \) peak is the lowest of the three. The area percentage of the \( \Delta \text{Di-2,6diS} \) peak is NLT 78.6%.

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: (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 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.
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.

- **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 100 mL of Solution C with stirring. Use within 24 h, and discards afterward.

- **Instrumental conditions**
  On *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: −72.0° 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°C 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

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