Chondroitin Sulfate Sodium

Type of Posting	Revision Bulletin
Posting Date	29–May–2015; updated 01–Jun–2015*
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Expert Committee	Monographs—Dietary Supplements and Herbal Medicines
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2010-2015 Council of Experts, the Monographs—Dietary Supplements and Herbal Medicines Expert Committee has revised the Chondroitin Sulfate Sodium monograph. The purpose for the revision is to add the use of an alternative enzyme, chondroitinase AC from *Flavobacterium heparium* to the test for the *Limit of Nonspecific Disaccharides* due to the unavailability /shortage of the chondroitinase AC from *Arthrobacter auresens* (Chromadex, part number ASB-00003613-10). In addition, several changes were made to clarify and improve the performance of the *Limit of Nonspecific Disaccharides* test.

The Chondroitin Sulfate Sodium Revision Bulletin supersedes the currently official monograph. This Revision Bulletin will be incorporated in the *USP 39–NF 34.*

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

* The Revision Bulletin Notice was updated on June 1, 2015, to correct the official date for the Revision Bulletin listed in the notice. The original posting on May 29, 2015 had the incorrect official date of July 1, 2015 listed on the Notice instead of June 1, 2015. The monograph that was posted on May 29, 2015 had the correct official date of June 1, 2015 and no changes were made to the text of the monograph.

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.]

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
- **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.
- **D. SPECIFIC ROTATION:** Meets the requirements for *Optical Rotation* ⟨781S⟩*, Specific Rotation* in *Specific Tests*

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 to 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
  Samples: *Standard solutions*, *Sample solution*, and *Diluent*
  Transfer 5.0 mL each of the *Standard solutions* 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%
- **CHLORIDE AND SULFATE ⟨221⟩,** *Chloride*: 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 ⟨221⟩,** *Sulfate*
  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.
- **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 that is constructed in such a way that any leakage of electrolyte will produce a short that 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 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 *Standard solution A*, *Standard solution B*, and *Sample solution* 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 V (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*.

Delete the following:

•• **HEAVY METALS,** *Method II* ⟨231⟩: NMT 20 ppm• (Official 1-Dec-2015)

SPECIFIC TESTS

Change to read:

- **LIMIT OF NONSPECIFIC DISSACCHARIDES**

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
4.5	100	0
21.0	61	39
21.1	100	0

Buffer solution: 50 mM tris(hydroxymethyl)aminomethane and 60 mM sodium acetate•• (RB 1-Jun-2015), adjusted with diluted hydrochloric acid to a pH of 8.0
Blank: Water
Chondroitinase AC solution: •Use appropriate chondroitinase AC that is capable of cleaving the *N*-acetylhexosaminide linkage in chondroitin 4-sulfate and chondroitin 6-sulfate, yielding Δ[4]-unsaturated disaccharides (ΔDi-0S, ΔDi-4S, and ΔDi-6S). The working concentration of the chondroitinase AC in *Buffer solution* must be sufficient for a complete digestion and meet the enzyme suitability requirement that follows. [NOTE—If Chondroitinase AC from *Arthrobacter auresens*[3] is used, 0.2 Units/mL in *Buffer solution* is a typical working concentration; if Chondroitinase AC from *Flavobacterium heparium*[4] is used, 3 Units/mL in *Buffer solution* is a typical working concentration. The working enzyme concentration may be increased if a complete digestion could not be achieved. The working enzyme aliquots should be stored at −20° when not in use for a period of time to avoid a decrease in the enzyme activity. A working enzyme solution is typically stable for 4 days when stored at 4°.]• (RB 1-Jun-2015)
Enzyme suitability: Dilute the digested *Standard solution* •and digested *Blank*• (RB 1-Jun-2015) (see *Analysis* section) (1 in 10), and measure the absorbance at 230 nm in 1-cm path cells. •Make correction with the diluted *Blank*.• (RB 1-Jun-2015)

[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).
[3] Chondroitinase AC from *Arthrobacter auresens*, Chromadex, part number ASB-00003613-10.
[4] Chondroitinase AC from *Flavobacterium heparium*, ≥200 units/mg protein, Sigma-Aldrich, catalog number E2039.

Calculate the absorptivity of 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 requirement: 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*.

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*, *Sample solution*, and *System suitability solution* (prepared as directed for *Samples* in the *Analysis*)
[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 •1.0• (RB 1-Jun-2015) between the ΔDi-4S and ΔDi-6S peaks, •*Standard solution*• (RB 1-Jun-2015)
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 articles being tested. Performance of this test is required only for the articles being tested not meeting the *Acceptance criteria*. 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*]
•• (RB 1-Jun-2015)

Analysis
Samples: *Blank*, *Standard solution*, *Sample solution*, and *System suitability solution*
In four separate vials, combine 4 volumes •(e.g., 800 µL)• (RB 1-Jun-2015) of *Chondroitinase AC solution* with 1 volume •(e.g., 200 µL)• (RB 1-Jun-2015) each of *Standard solution*, *Sample solution*, *System suitability solution*, and *Blank*. Mix thoroughly. Incubate at 37° for 3 h. • [NOTE—the incubation period may be increased, if necessary, to complete the digestion.]• (RB 1-Jun-2015) Allow the solutions to cool before injection.
Calculate the percentage of specific disaccharides in the portion of Chondroitin Sulfate Sodium 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%

- **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: NMT 0.35
- **OPTICAL ROTATION ⟨781S⟩, *Specific Rotation***
  Sample solution: 30 mg/mL
  Acceptance criteria: –20.0° to –30.0°
- **pH ⟨791⟩:** 5.5–7.5, in a solution (1 in 100)
- **LOSS ON DRYING ⟨731⟩**
  [NOTE—Chondroitin Sulfate Sodium is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]
  Analysis: Dry at 105° for 4 h.
  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, whether bovine, porcine, avian, or a mixture of any of them.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Chondroitin Sulfate Sodium RS