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Chondroitin Sulfate Sodium

Chondroitin, hydrogen sulfate, sodium salt

CAS RN[®]: 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

Change to read:

- **A.** [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K ▲](#) (CN 1-MAY-2020)
- **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 (Δ Di-4S), dehydrated glucuronic acid-[1→3]-chondrosamine-6-sulfated (Δ Di-6S), and nonsulfated dehydrated glucuronic acid-[1→3]-chondrosamine (Δ Di-0S) in the enzymatically digested *Standard solution*. By peak-area response, Δ Di-4S is the most abundant, followed by Δ Di-6S, with Δ Di-0S being the least abundant of the three. The ratio of the peak response of the Δ Di-4S to the Δ Di-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% on the dried basis
- **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 membranes¹ (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 applicator² 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 [Ultraviolet-Visible Spectroscopy \(857\)](#).)

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