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Glucosamine and Chondroitin Sulfate Sodium Tablets

DEFINITION

Glucosamine and Chondroitin Sulfate Sodium Tablets are prepared from either Glucosamine Hydrochloride, Glucosamine Sulfate Sodium Chloride, Glucosamine Sulfate Potassium Chloride, or a mixture of any of them, with Chondroitin Sulfate Sodium. Tablets contain NLT 90.0% and NMT 120.0% of the labeled amounts of chondroitin sulfate sodium and glucosamine ($C_6H_{13}NO_5$).

[Note—Chondroitin Sulfate Sodium is extremely hygroscopic once dried. Avoid exposure to atmosphere, and weigh promptly.]

IDENTIFICATION

• **A.** The retention time of the major peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the test for *Content of Glucosamine*.

• **B. Electrophoresis**

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: 0.1% (w/v) toluidine blue in 0.1 M acetic acid

Standard solution: Use the *Standard solution* of middle concentration from the test for *Content of Chondroitin Sulfate Sodium*.

Sample solution: Prepare as directed in the test for *Content of Chondroitin Sulfate Sodium*.

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* and *Standard 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.

Acceptance criteria: The principal spot of the *Sample solution* has the same migration as the principal spot of the *Standard solution*. [Note—Document the results by taking a picture within 15 min of completion of destaining.]

STRENGTH

• **Content of Glucosamine**

Diluent: Transfer 29 µL of acetic acid and 5 mL of acetonitrile to a 100-mL volumetric flask containing 50 mL of water. Dilute with water to volume.

Borate buffer: 0.2 M (76.3 g/L of sodium borate in water) adjusted with hydrochloric acid TS to a pH of 9.5

Acetate buffer: 6.80 g/L of sodium acetate trihydrate in water adjusted with dilute acetic acid to a pH of 5.9

Derivatizing reagent: In a 14-mL polypropylene culture tube, dissolve 50 mg of *o*-phthalaldehyde in 1.25 mL of anhydrous methanol. Add 50 µL of 3-mercaptopropionic acid and 11.2 mL of *Borate buffer*, and mix gently. Allow to stand in the dark for 30 min before use. [Note—Reagent strength is maintained by adding 10 µL of 3-mercaptopropionic acid every 2 days. Storage should be in the dark at room temperature, and can be used for NMT 2 weeks.]

Mobile phase: Methanol and *Acetate buffer* (1:9)

Standard solution: 1.0 mg/mL of USP Glucosamine Hydrochloride RS in water. Allow to stand at room temperature for 1 h.

Sample solution: Transfer an equivalent to 25 mg of glucosamine, from finely powdered Tablets (NLT 20), to a 25-mL volumetric flask. Dilute with *Diluent* to volume. Mix on a vortex mixer to suspend the powder in solution. Sonicate in a 65° water bath for 20 min. Remove from the bath, stir for 5 min with the aid of a magnetic stirrer, and centrifuge.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

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Detector: UV 340 nm

Column: 3.0-mm × 5-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: Five individual aliquots of the *Standard solution* derivatized as directed in the *Analysis*. Each derivatized aliquot is injected only once.

[NOTE—The relative retention times for the β-anomer and the α-anomer are 1.0 and 1.8, respectively. The retention time for the β-anomer is NLT 4 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% from five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer 100 µL of the *Derivatizing reagent* and 100 µL of the *Standard solution* or *Sample solution* to a vial containing 400 µL of *Borate buffer*. Allow the derivatization to proceed for 1 min. Inject the derivatized solutions immediately after the derivatization reaction.

Calculate the percentage of the labeled amount of glucosamine ($C_6H_{13}NO_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the β-anomer from the derivatized *Sample solution*

r_S = peak response of the β-anomer from the derivatized *Standard solution*

C_S = concentration of USP Glucosamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of glucosamine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of glucosamine, 179.17

M_{r2} = molecular weight of glucosamine hydrochloride, 215.63

Acceptance criteria: 90.0%–120.0%

• **CONTENT OF CHONDROITIN SULFATE SODIUM**

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 approximately 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.

Standard solutions: 1.5, 1.0, and 0.5 mg/mL of USP Chondroitin Sulfate Sodium RS in water

Sample solution: Transfer an equivalent to 100 mg of chondroitin sulfate sodium, from finely powdered Tablets (NLT 20), to 60 mL of water. Shake to suspend the powder in solution. Sonicate in a 65° water bath for 20 min. Remove from the bath, and stir or shake for 5 min. Dilute with water to 100 mL, and centrifuge or pass through a suitable filter.

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* and *Sample solution*

Transfer 5.0 mL of each *Standard solution* and the *Sample solution* to separate titration vessels. Add 25 mL of *Diluent* to each. Stir until a steady reading is obtained with a photoelectric probe 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 the labeled amount of chondroitin sulfate sodium in the portion of Tablets taken:

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

C = determined concentration of chondroitin sulfate sodium in the *Sample solution* (mg/mL)

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