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Glucosamine, Chondroitin Sulfate Sodium, and Methylsulfonylmethane Tablets

DEFINITION

Glucosamine, Chondroitin Sulfate Sodium, and Methylsulfonylmethane 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 and Methylsulfonylmethane. Tablets contain NLT 90.0% and NMT 120.0% of the labeled amounts of chondroitin sulfate sodium and glucosamine ($C_6H_{13}NO_5$) and NLT 90.0% and NMT 110.0% of the labeled amount of methylsulfonylmethane ($C_2H_6O_2S$).

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

IDENTIFICATION

• **A. PRESENCE OF GLUCOSAMINE:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Glucosamine*.

• **B. PRESENCE OF CHONDROITIN SULFATE**

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 *Content of Chondroitin Sulfate Sodium*.

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

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 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 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 *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 band from the *Sample solution* has the same migration as the principal band from the *Standard solution*.

[NOTE—Document the results by taking a picture within 15 min of completion of destaining.]

• **C. PRESENCE OF METHYLSULFONYLMETHANE:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Content of Methylsulfonylmethane*.

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, and 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. [NOTE—Buffer must be stored at room temperature and can be warmed to dissolve if crystallization occurs.]

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 NLT 20 Tablets, finely powdered, to a 25-mL volumetric flask, and 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**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 *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% for 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 the *Sample solution* to a vial containing 400 µL of *Borate buffer*, and 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% of the label claim**• 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 an equivalent to 100 mg of chondroitin sulfate sodium from NLT 20 Tablets, finely powdered, to 60 mL of water, and shake to suspend the powder in solution. Sonicate in a 65° water bath for 20 min. Remove from the bath, stir or shake for 5 min, dilute with water to 100 mL, and centrifuge or pass through a suitable filter.

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.

Titrimetric system(See [Titrimetry \(541\)](#).)**Mode:** Photometric titration**Titrant:** 1 mg/mL of cetylpyridinium chloride in water**Endpoint detection:** Turbidimetric with photoelectric probe

Analysis: Transfer 5.0 mL of each *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 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 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)

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

Acceptance criteria: 90.0%–120.0% of the label claim**• CONTENT OF METHYLSULFONYLMETHANE**

Diluent: Transfer 950 mL of methanol to a 1-L volumetric flask. Add 0.60 mL of diethylene glycol methyl ether, and dilute with methanol to volume.

Standard solution: 0.4 mg/mL of [USP Methylsulfonylmethane RS](#) in *Diluent*. Sonicate at 50° for 1 min, and allow to cool to room temperature.