

Glucosamine and Chondroitin Sulfate Sodium Tablets

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 not less than 90.0 percent and not more than 120.0 percent 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.

Packaging and storage— Preserve in tight, light-resistant containers.

Labeling— The label indicates the types of glucosamine salts contained in the article and the species source from which chondroitin was derived. Label it to state the source(s) of chondroitin sulfate sodium, whether bovine, porcine, avian, or a mixture of any of them. The label states on the front panel the content of chondroitin sulfate sodium on the dried basis.

Identification—

A: The retention times of the major peaks in the chromatogram of the Test solution correspond to those in the chromatogram of the Standard solution, as obtained in the test for Content of glucosamine (presence of glucosamine).

B: Standard solution and Test solution— Prepare as directed in the test for Content of chondroitin sulfate sodium under [Chondroitin Sulfate Sodium Tablets](#).

Procedure (see [Electrophoresis](#) [726](#))— Proceed as directed for Identification under [Chondroitin Sulfate Sodium Tablets](#) (presence of chondroitin sulfate).

[Disintegration and dissolution](#) [2040](#) : meet the requirements for Dissolution.

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amount of glucosamine ($C_6H_{13}NO_5$) dissolved by employing the following method. Diluent, 0.2 M Borate buffer, Derivatizing reagent, Mobile phase, and Chromatographic system— Proceed as directed in the test for Content of glucosamine.

Standard solution— Prepare as directed in the test for Content of glucosamine. Dilute with a suitable quantity of water, if necessary.

Test solution— Use the solution under test.

Procedure— Proceed as directed in the test for Content of glucosamine. Calculate the quantity, in mg, of glucosamine ($C_6H_{13}NO_5$) dissolved by the formula:

$$(179.17/215.63)(900C)(r_U / r_S)$$

in which the terms are as defined therein.

Tolerances— Not less than 75% of the labeled amount of $C_6H_{13}NO_5$ is dissolved in 60 minutes. Determine the amount of chondroitin sulfate sodium dissolved by employing the following method.

Cetylpyridinium chloride solution, Diluent, Standard solutions, and Test solution— Prepare as directed in the test for Content of chondroitin sulfate sodium under [Chondroitin Sulfate Sodium Tablets](#).

Procedure— Proceed as directed in the test for Content of chondroitin sulfate sodium under [Chondroitin Sulfate Sodium Tablets](#), adjusting the volume of the sample and/or the concentrations of the standards, if necessary. Calculate the quantity, in mg, of chondroitin sulfate sodium dissolved by the formula: $900C$

in which C is the concentration, in mg per mL, of chondroitin sulfate sodium in the solution under test.

Tolerances— Not less than 75% of the labeled amount of chondroitin sulfate sodium is dissolved in 60 minutes.

[Weight variation](#) [2091](#) : meet the requirements.

Content of glucosamine—

Diluent— Transfer 29 μ L of acetic acid and 5 mL of acetonitrile to a 100-mL volumetric flask containing about 50 mL of water, and dilute with water to volume.

0.2 M Borate buffer— Dissolve 7.63 g of sodium borate in 80 mL of water, and adjust with hydrochloric acid TS to a pH of 9.5. Transfer to a 100-mL volumetric flask, dilute with water to volume, and mix. [note—Buffer must be stored at room temperature and can be used indefinitely, but must 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 0.2 M Borate buffer, and mix gently. Allow to stand in the dark for 30 minutes before use. [Note—Reagent strength is maintained by adding 10 µL of 3-mercaptopropionic acid every two days. Storage should be in the dark, at room temperature, and can be used for not more than 2 weeks.]

Mobile phase— In a 1000-mL volumetric flask dissolve 6.80 g of sodium acetate trihydrate in 700 mL of water. Adjust with dilute acetic acid to a pH of 5.9, dilute with water to volume, and mix. Combine 100 mL of methanol with 900 mL of acetate buffer, and mix thoroughly. Pass through a nylon membrane filter having a 0.45-µm or finer porosity, and degas. Make adjustments if necessary.

Standard solution— Dissolve an accurately weighed quantity of [USP Glucosamine Hydrochloride RS](#) in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 1.0 mg per mL. Allow to stand at room temperature for 1 hour.

Test solution— Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of glucosamine, 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°C water bath for 20 minutes. Remove from the bath, stir for 5 minutes with the aid of a magnetic stirrer, and centrifuge.

Chromatographic system— The liquid chromatograph is equipped with a 340-nm detector and a 3.0-mm × 5-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph five individual aliquots of the Standard solution derivatized as directed for Procedure. Each derivatized aliquot is injected only once. The relative retention times are 1.0 for the β-anomer and 1.8 for the α-anomer, and the typical retention time of the β-anomer is not less than 4 minutes. The relative standard deviation calculated from these five replicates is not more than 2.0%.

Procedure— Transfer 100 µL of the Derivatizing reagent and 100 µL of the Standard solution or the Test solution to a vial containing 400 µL of 0.2 M Borate buffer, mix, allow the derivatization to proceed for 1 minute, and inject the derivatized solution into the chromatograph. Separately

inject equal volumes (about 10 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

[Note—Inject the derivatized solution immediately after the derivatization reaction.] Calculate the quantity, in mg, of glucosamine ($\text{C}_6\text{H}_{13}\text{NO}_5$) in the portion of Tablets taken by the formula:

$$(179.17/215.63)(100C)(r_U / r_S)$$

in which 179.17 and 215.63 are the molecular weights of glucosamine and glucosamine hydrochloride, respectively; C is the concentration, in mg per mL, of [USP Glucosamine Hydrochloride RS](#) in the Standard solution; and r_U and r_S are the peak responses for the β -anomer obtained from the Test solution and the Standard solution, respectively.

Content of chondroitin sulfate sodium—

Cetylpyridinium chloride solution, Diluent, Standard solutions, Test solution, and Procedure—

Proceed as directed in the test for Content of chondroitin sulfate sodium under [Chondroitin Sulfate Sodium Tablets](#).