Suitability requirements
Tailing factor: NMT 2.0 for the glucosamine peak
Efficiency: NLT 1500 theoretical plates
Relative standard deviation: NMT 2.0%.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of glucosamine sulfate potassium chloride [$(C_6H_{14}NO_5)_2SO_4 \cdot 2KCl$] in the portion of Glucosamine Sulfate Potassium Chloride taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glucosamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Glucosamine Sulfate Potassium Chloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of glucosamine sulfate potassium chloride, 605.52
M_{r2} = twice the molecular weight of glucosamine hydrochloride, 431.26
Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

- **CONTENT OF SULFATE**
Sample: 1 g of Glucosamine Sulfate Potassium Chloride
Analysis: Transfer the *Sample* to a 250-mL beaker, and dissolve in 100 mL of water. Add 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to completely precipitate the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 h. Pass the mixture through ashless filter paper. Transfer the residue quantitatively to a new filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Calculate the content of sulfate by multiplying the weight obtained by 0.4116.
Acceptance criteria: 15.5%–16.5%

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** 26.5%–31.0%
- **SODIUM:** A solution (1 in 10), tested on a platinum wire, does not impart a pronounced yellow color to a nonluminous flame.
- **ARSENIC,** *Method II* ⟨**211**⟩**:** NMT 3 µg/g
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
Sample solution: 35 mg/mL. Measure the specific rotation 3 h after preparation.
Acceptance criteria: +47.0° to +53.0°
- **pH** ⟨**791**⟩
Sample solution: 20 mg/mL
Acceptance criteria: 3.0–5.0
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Glucosamine Hydrochloride RS

Glucosamine Sulfate Sodium Chloride

$(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$ 573.31
Bis(D-glucose, 2-amino-2-deoxy-), sulfate sodium chloride complex;
Bis(2-amino-2-deoxy-β-D-glucopyranose) sulfate sodium chloride complex (–,–) [38899-05-7].

DEFINITION

Glucosamine Sulfate Sodium Chloride contains NLT 98.0% and NMT 102.0% of glucosamine sulfate sodium chloride [$(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$], calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
Sample: Transfer 50 mg of Glucosamine Sulfate Sodium Chloride to a centrifuge tube, and dissolve in 2 mL of water. Add 0.5 mL of barium chloride TS, and centrifuge. Collect the supernatant, and evaporate to dryness. Dry the residue at 105° for 2 h.
Acceptance criteria: The IR spectrum of the *Sample* matches that of a similar preparation of USP Glucosamine Hydrochloride RS, except that the addition of barium chloride TS is omitted.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩ and *Sodium* ⟨**191**⟩**:** Meets the requirements
- **C.** The retention time of the glucosamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **D. SULFATE:** In the test for *Content of Sulfate*, after the addition of barium chloride TS a white precipitate is formed.

ASSAY

- **PROCEDURE**
Buffer: In a 1-L volumetric flask, dissolve 3.5 g of dibasic potassium phosphate in water, add 0.25 mL of ammonium hydroxide, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 7.5.
Mobile phase: Acetonitrile and *Buffer* (75:25)
Diluent: Acetonitrile and water (50:50)
Standard solution: 3.8 mg/mL of USP Glucosamine Hydrochloride RS in *Diluent*. Shake for 5 min by mechanical means to completely dissolve.
Sample solution: Transfer 250 mg of Glucosamine Sulfate Sodium Chloride to a 50-mL volumetric flask. Dissolve in 30 mL of *Diluent,* and shake by mechanical means. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The peak for the glucosamine moiety elutes at about 10 min. The chromatogram shows additional peaks near the void volume, due to the counter ions.]

Suitability requirements
Tailing factor: NMT 2.0 for the glucosamine peak
Efficiency: NLT 1500 theoretical plates
Relative standard deviation: NMT 2.0%.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of glucosamine sulfate sodium chloride [$(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$] in the portion of Glucosamine Sulfate Sodium Chloride taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glucosamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Glucosamine Sulfate Sodium Chloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of glucosamine sulfate sodium chloride, 573.31
M_{r2} = twice the molecular weight of glucosamine hydrochloride, 431.26
Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

- **CONTENT OF SULFATE**
Sample: 1 g of Glucosamine Sulfate Sodium Chloride
Analysis: Transfer the *Sample* to a 250-mL beaker, and dissolve in 100 mL of water. Add 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to completely precipitate the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 h. Pass the mixture through ashless filter paper. Transfer the residue quantitatively to a new filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Calculate the content of sulfate by multiplying the weight obtained by 0.4116.
Acceptance criteria: 16.3%–17.3%

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** 22.5%–26.0%
- **ARSENIC,** *Method II* **⟨211⟩:** NMT 3 µg/g
- **POTASSIUM**
Analysis: Acidify 5 mL of a solution (1 in 20) with 6 N acetic acid, and add 5 drops of sodium cobaltinitrite TS.
Acceptance criteria: No precipitate is formed.
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* **⟨781S⟩**
Sample solution: 35 mg/mL. Measure the specific rotation 3 h after preparation.
Acceptance criteria: +50.0° to +55.0°
- **pH ⟨791⟩**
Sample solution: 20 mg/mL
Acceptance criteria: 3.0–5.0
- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Glucosamine Hydrochloride RS

Glucosamine and Methylsulfonylmethane Tablets

DEFINITION

Glucosamine 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 Methylsulfonylmethane. Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of glucosamine ($C_6H_{13}NO_5$) and NLT 90.0% and NMT 110.0% of the labeled amount of methylsulfonylmethane ($C_2H_6O_2S$).

IDENTIFICATION

- **A. PRESENCE OF GLUCOSAMINE:** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Content of Glucosamine*.
- **B. 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. [NOTE—Buffer must be stored at room temperature. It must be warmed to dissolve if crystallization occurs.]
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 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 for *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.]