

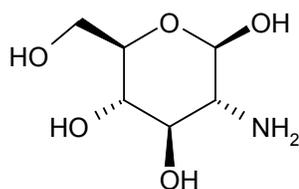
### 0049 - Glucosamine by HPLC

**Chemical Names:** 2-Amino-2-deoxy-D-glucose; (2R,3R,4R,5S,6R)-3-amino-6-hydroxymethyl-tetrahydropyran-2,4,5-triol

**Common Names:** Chitosamine

**Molecular Weight:** R179.17

**Chemical Formula:** C<sub>6</sub>H<sub>13</sub>NO<sub>5</sub>



Glucosamine

#### Solubility:

Highly water soluble; slightly soluble in cold methanol and ethanol; insoluble in ether, chloroform, benzene, and other nonpolar organic solvents.

#### Other Physical/Chemical Data:

$\beta$ -Form:  $[\alpha]_D = +28^\circ$  at 20°C (c = 1); melting point = 110°C (decomposition)

$\alpha$ -Form:  $[\beta]_D = +100^\circ$  at 20°C (c = 1)

NOTE: Both  $\alpha$ - and  $\beta$ -forms change to  $[\alpha]_D +47.5^\circ$  over time.

#### Uses:

Glucosamine has been used to reduce the pain associated with osteoarthritis, a condition that causes progressive breakdown of the cartilage in joints. It is usually available commercially as either the hydrochloride or sulfate salt. Glucosamine is sometimes combined with chondroitin sulfate in dietary supplements.

#### Modes of Action:

The mode of action of glucosamine is not completely understood. It seems to be related to the biosyntheses or activation of glycosaminoglycans.<sup>1,2</sup>

#### Methods of Analysis

Glucosamine is a highly polar molecule lacking a UV chromophore. As a result, methods of analysis usually involve either precolumn derivatization followed by reversed-phase HPLC or ion-exchange or ion-pair chromatography followed by either refractive index (RI) or electrochemical (EC) detection.

Although the mode of action and efficacy of glucosamine hydrochloride and glucosamine sulfate are believed to be the same, the potency is not due to the differences in molar weight between the two forms. Glucosamine free base constitutes 83.1% by weight of glucosamine hydrochloride, whereas it constitutes only 78.5% by weight of the hemisulfate form, and this amount may be even less as the hemisulfate salt often contains potassium chloride as well. Specifications and labels of glucosamine products should be examined to determine if the product is the free base or one of the salts.

### Method 1:

The Institute for Nutraceutical Advancement (INA) has developed a method<sup>3</sup> for the analysis of glucosamine in raw materials utilizing precolumn derivatization with phenylisothiocyanate followed by reversed-phase HPLC. This method can be adapted easily to finished products containing glucosamine.

#### *Standard and Sample Preparation:*

Weigh about 40 mg of glucosamine standard or sample (calculated as the free base), transfer to a 50-mL volumetric flask, and dissolve in 0.1 M sodium acetate solution. Pipet 5 mL of this solution into a 50-mL volumetric flask, and add 400  $\mu$ L of phenylisothiocyanate and 15 mL of methanol. Fill to volume with methanol–water (60:40), and transfer 5 mL of this solution to a reaction vial, cap, and heat at 80°C for 15 minutes. Cool, and extract the solution with 5 mL of hexane to remove unreacted phenylisothiocyanate.

#### *Chromatography:*

Column: Phenomenex Luna C18, 150  $\times$  4.6 mm.  
Mobile phase: Acetonitrile–water–phosphoric acid (10:90:0.1).  
Flow rate: 1.5 mL/minute  
Injection volume: 10  $\mu$ L  
Detection wavelength: UV at 240 nm  
Run time: 10 minutes

#### *Validation Data:*

Not available

### Method 2:

Way et al.<sup>4</sup> developed an ion-pair HPLC method utilizing refractive index detection for the determination of glucosamine in dietary supplements.

#### *Sample Preparation:*

Weigh an amount of sample equivalent to about 100 mg of glucosamine into a 100-mL volumetric flask and dilute to volume with mobile phase. Sonicate the samples for approximately 20 minutes to dissolve the glucosamine.

#### *Chromatography:*

Column: MetaChem Inertsil ODS-3, 250  $\times$  4.6 mm.  
Mobile phase: 10% methanol–90% 0.005 M octanesulfonate, pH 2.1.  
Flow rate: 1.0 mL/minute  
Injection volume: 50  $\mu$ L  
Detection: Refractive index at 40°C

**Validation Data:**

Linearity: Correlation coefficient >0.999 (52% to 210% of target).

Accuracy: Average recovery = 98.4%.

Precision: 2.13% RSD of 12 sample preparations.

Selectivity: Interferences in placebo <1% of target, verified by PDA.

Ruggedness: Average results on second day were 97.5% of first-day results.

Robustness: Not specified

LOD/LOQ: Not specified

**Method 3:**

Another method using precolumn derivatization with phenylisothiocyanate followed by reversed-phase HPLC was developed by Liang et al.<sup>5</sup> The method differs from the INA method in both sample preparation and chromatography.

**Standard and Sample Preparation:**

Prepare glucosamine standard solutions in water at concentrations ranging from about 6.6 to 16.6 mcg/mL. Prepare samples in water so that the glucosamine concentration is within the linearity range. To these solutions, add 250 µL of 0.3 M phosphate buffer (pH 8.0) and 250 µL of 5% phenylisothiocyanate. Incubate the solutions at room temperature for 10 minutes, and then evaporate to dryness at 50°C under nitrogen. Reconstitute the samples and standards in 200 µL of mobile phase.

**Chromatography:**

Column: Phenomenex Bondclone C18, 300 × 3.9 mm.

Mobile phase: Methanol–water–acetic acid (10:89.96:0.04).

Flow rate: 1.2 mL/minute

Injection volume: 20 µL

Detection wavelength: UV at 254 nm

Run time: 10 minutes

**Validation Data:**

Linearity: Correlation coefficient >0.99 (6.65 to 16.63 mcg/mL).

Accuracy (intraday): +0.90% to –2.54% relative error.

Accuracy (interday): –0.75% to 2.70% relative error.

Precision (intraday): 0.47% to 4.18% RSD.

Precision (interday): 0.99% to 3.18% RSD.

Selectivity: Not specified

Robustness: Determined using two different lots of the same analytical column and two different batches of mobile phase (only retention times compared).

LOD/LOQ: Not determined

## References:

1. Lippiello L, Woodward J, Karpman R, et al. Beneficial effect of cartilage disease-modifying agents testing in chondrocyte cultures and a rabbit instability model of osteoarthritis. *Arthritis Rheum*. 1999;42(Suppl.):S256.
2. Runkel DR, Cupp MJ. Glucosamine sulfate use in osteoarthritis. *Am J Health Syst Pharm*. 1999;56:267
3. Institute for Nutraceutical Advancement method 121.000, panax and American ginseng assay by HPLC. <http://www.nsfina.org/business/ina/index.asp?program=INA>.
4. Way WK, Gibson KG, Breite AG. Determination of glucosamine in nutritional supplements by reversed-phase ion-pairing HPLC. *J Liq Chromatogr Relat Technol*. 2000;23(18):2831-71.
5. Liang Z, Leslie J, Adebowale A, et al. Determination of the nutraceutical, glucosamine hydrochloride, in raw materials, dosage forms and plasma using pre-column derivatization with ultraviolet HPLC. *J Pharm Biomed Anal*. 1999;20:807-14.