Spectrophotometric Method for Determination of Glucosamine in Tablets

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A rapid and sensitive method has been developed for the determination of glucosamine sulphate from tablets by UV-spectrophotometery. In this method, glucosamine sulphate was reacted with phenylisothiocyanate in presence of a base to yield phenylthiourea derivative. This derivative showed maximum absorbance at 240 nm. Beer's law is obeyed in the concentration range of 5-25 μg/ml. The method was validated in terms of linearity, precision (relative standard deviation 1.1%), accuracy and specificity. The proposed method is the only method available for spectrophotometric determination of the drug. It is simple, precise, accurate, sensitive and reproducible and can be used for the routine quality control testing of the marketed formulations.

Glucosamine sulphate (GLS) is a water-soluble amine sugar that is extensively used in the treatment of various conditions of arthritis and collagen deficiency[1-4]. Literature survey reveals that the drug can be estimated only by HPLC[5] and no spectrophotometric methods have been reported. The present study describes a simple, sensitive, accurate and precise spectrophotometric method for estimation of GLS in tablet formulation.

The reference standard of GLS was procured as a gift sample from M/s Meyer Organics Ltd., Thane. Phenylisothiocyanate (PITC) AR grade was purchased from Fluka, Switzerland. All the other chemicals and solvents used were of AR grade. UV SL-159 Elico make spectrophotometer was used for the studies.

A standard solution of GLS (1000 μg/ml) was prepared in 0.1 M aqueous sodium acetate solution. (S1). Ten tablets (Cartilamine® 500 mg, Troikaa, Ahmedabad; and Cartisafe Forte® 500 mg Jenburkt, Mumbai) were weighed and finely powdered. The powder equivalent to GLS (100 mg) was dissolved in 50 ml of 0.1 M aqueous sodium acetate solution. The solution was then filtered and the residue was washed thoroughly with 0.1 M aqueous sodium acetate solution. The filtrate and the washings were combined in a 100 ml volumetric flask and diluted to the mark with 0.1 M aqueous sodium acetate solution (T1). The solution of GLS should be prepared 24 h before the analysis.

For the preparation of phenylthiourea (PTH) derivative, an aliquot of 4 ml of the standard solution of GLS (S1) was transferred to a 25 ml volumetric flask and 0.4 ml PITC along with 15 ml methanol was added. The volume was made up to the mark with 60% aqueous methanol (S2). An aliquot of 10 ml of S2 was transferred to a calibrated test tube and was heated for 20 min. in a boiling water bath. The test tube was cooled and the volume was made to 10 ml with distilled water. The solution was made free of unreacted PITC by extraction with diethyl ether (two portions of 15 ml each) and the aqueous layer containing PTH derivative of GLS was collected. An aliquot of 5 ml of this aqueous layer was transferred to a 50 ml volumetric flask and the volume was made up to the mark with distilled water (S3). The sample solution T1 was treated in the same manner as that of the standard to obtain T3.

For the calibration curve, a series of dilutions of S3 were prepared in distilled water so as to obtain the concentrations in the range of 5-25 μg/ml. The absorbance was measured on UV/Vis spectrophotometer at 240 nm against reagent blank. For quantification of GLS in the tablet formulations, an aliquot of 2 ml of T3 was diluted to 10 ml with distilled water and the absorbance was measured at 240 nm on UV/Vis spectrophotometer. The concentration of GLS present was deduced from the calibration curve.

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The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, specificity and repeatability. The limit of detection and limit of quantification were also determined. Optimum operating conditions used in the procedures were established adopting variation of one variable at a time. The optical characteristics of the method are presented in Table 1. Measuring six replicate samples of the drug in Beer's law limit tested the precision and accuracy of the method. The accuracy of the method was further checked by recovery experiments, which were performed by standard addition method.

Literature survey on the analytical methods for GLS estimation had revealed only the HPLC[5] methods, which are time consuming and costly. The proposed method was found to be cost-effective and can thus be utilized in routine analysis.

The aqueous solution of the drug exhibited absorption maxima at 190 nm, which cannot be utilized for the quantitative determinations. An alternative approach was to derivatize the drug in order to insert the chromophore in the molecule so as to enable the spectrophotometric estimation. The amino group of the drug was reacted with PITC to prepare the PTH derivative, which exhibited absorption maxima at 240 nm. The method was then optimized for the reaction conditions and it was observed that the reaction is effected by heating the sample in alkaline medium with the methanolic solution of PITC. Further, the drug, being an amino sugar, requires a stabilization period of 24 h in alkaline pH, which is provided by sodium acetate. The content of GLS in tablet formulation was calculated by applying suitable dilution factor and extrapolating from the calibration curve. The content of GLS in the tablets was found to be 499.8±0.07 and 499.0±0.09 mg (Table 2).

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION OF THE PROPOSED METHOD.

Parameter for PTH derivative	Observations
λ_{max} (nm)	240
Beer's Law limit (µg/ml)	1.5-15 (for PTH
derivative 5-25)	
Molar absorptivity (lit/mol/cm)	1.93 x 10^4
Sandell's sensitivity	
(µg/ml/cm^2/0.001 absorbance unit)	0.0230
Regression Equation (Y =a+bx)*	0.1069 x + 0.0407
Slope (b)	1.069 x 10^{-1}
Intercept (a)	4.07 x 10^{-2}
Coefficient of Correlation	0.9992
Relative standard deviation (%)**	1.1
% Range of errors (confidence limits)**	0.265
0.01 level	

*Y=a+bx, where x is the concentration of the analyte and Y is absorbance unit, **Average of six determinations.

TABLE 2: ANALYSIS OF TABLET FORMULATIONS CONTAINING GLS

Formulation	Labeled value (mg)	Amount Found by the Proposed Method	% Recovery by the Proposed Method*
Tablet 1	500	499.8±0.07	99.8±0.04
Tablet 2	500	499.0±0.09	99.6±0.15

Tablet 1 is Cartilamine, 500 mg manufactured by Troikaa, Ahmedabad and Tablet 2 is Cartisafe Forte, 500 mg Jenburkt, Mumbai. *Recovery of 10 mg added to the preanalyzed pharmaceutical dosage form (average of three determinations).

Linearity range for the PTH derivative was found to be 5-25 µg/ml with a correlation coefficient of 0.9992. The average linear regression equation was represented as y=0.1069x+0.0407, where x is concentration of GLS and y is absorbance. Accuracy of the method was evaluated by calculating the recovery of GLS by standard addition of 10 mg to the pre-analyzed pharmaceutical dosage form. The percentage recovery was found to be 99.8±0.04 and 99.6±0.15 (Table 2), ensuring the accuracy of the method. The different validation parameters for the proposed method are summarized in Table 1. The results indicate that the proposed method is simple, rapid, sensitive, precise and accurate for quantification of GLS from the formulations.

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