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Method Development and Validation for Glucosamine and Chondroitin Sulphate in Soflet Form by RP-HPLC.

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ABSTRACT

To develop a simple, economic, accurate reverse phase isocratic RP-HPLC simultaneous estimation method for the Glucosamine(500mg) and Chondroitin Sulphate(400mg) in Soflet dosage form. Extended-C18 (YMC pack ODS-250 x 4.6 mm, packed with 5 micron) in an isocratic mode with mobile phase Octane sulphonic acid in water : Acetonitrile : triethylamine (90.65 : 8.96 : 0.381) was used and adjusted the pH 4.0 with ortho phosphoric acid. The flow rate was 1.0ml/ min and effluent was monitored at 195 nm. The retention times were 1.72 min and 1.353 min for Glucosamine and Chondroitin Sulphate respectively. The linearity range was found to be 80 to 120%. The proposed method was validated. The reports was expressed that the proposed method was found to be simple, precise, accurate and rapid for determination of Glucosamine(500mg) and Chondroitin Sulphate(400mg) in Soflet dosage form.

INTRODUCTION

Glucosamine is a synthetic corticosteroid drug that is particularly effective as an anti-arthritic. Glucosamine is naturally present in the shells of Shellfish, animal bones and bone marrow. Even it is present in some fungi, such as Aspergillus niger[1]. Chondroitin Sulphate is chemically known as polymeric D-galactosamine and D-glucuronic acid. Chondroitin sulfate supplements are nothing but isomeric mixtures of chondroitin sulfate A and chondroitin sulfate C and are derived from cartilaginous rings of cow trachea and pork by products (such as ears and snout). It is used to relief the pain of osteoarthritic joints and acts as an anti-inflammatory activity (Anti-arthritic)[2]. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analytes to be more specific, accurate and precise. The main objective for developing method is to improve the conditions and parameters, which should be followed in the development and validation. A survey of literature reveals that good simultaneous analytical methods are not available for the drugs Glucosamine and Chondroitin Sulphate. Even though very few methods of individual estimation of above drugs are available. The existing physicochemical methods are inadequate to meet the requirements for the simultaneous determination; hence it is proposed to develop new methods for the assay of Glucosamine (500mg) and Chondroitin Sulphate(400mg) in Soflet dosage form adapting available analytical technique like high performance liquid chromatography (HPLC). According to the literature survey it was found that few analytical methods such as HPLC and UV-Visible analysis were reported for the estimation of Glucosamine and Chondroitin Sulphate individually[3,4]. The objective of the proposed method is to develop simple and accurate methods for the determination of Glucosamine and Chondroitin Sulphate RP-HPLC methods in soflet dosage forms.

MATERIALS AND METHODS

Glucosamine and Chondroitin Sulphate were obtained from Geltec Pvt, Ltd. (Geltec Pvt, Ltd. India). A commercial sample of soflet consist Glucosamine (500mg) and Chondroitin Sulphate (400mg) were procured from Geltec Pvt, Ltd., and were used within their shelf-life period. The HPLC grade Acetonitrile and water from Rankem (New Delhi, India) and all other chemicals used were of pharmaceutical or analytical grade from E-Merck. HPLC grade water was prepared using Millipore purification system.

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Quantitative HPLC was performed on Agilent HPLC (QC-62 RRLC-01), 1200 series with UV-Visible detector. Extended-C18 (YMC pack ODS-250 x 4.6 mm, packed with 5 micron) column used for the chromatographic separation. Automatic injections (20 µl) were used.

Trial and error method

The analytical wavelength was set at 195 nm which was selected using scan mode in UV visible detector from 190 nm to 400 nm using mentioned mobile phase. 195nm was selected for the estimation based on the overlap spectra and its solvent effect. To develop a suitable and robust HPLC method for the determination of Glucosamine and Chondroitin Sulphate, different mobile phases Acetonitrile : water, were used in different compositions of mobile phases at different flow rates. Finally, the mobile phase Octane sulphonic acid in water: Acetonitrile : triethylamine at the ratio of 90.65 : 8.96 : 0.381(adjusted the pH 4.0 with ortho phosphoric acid) at a flow rate of 1.0 ml/ min gave peaks good resolution for Glucosamine and Chondroitin Sulphate. Glucosamine and Chondroitin Sulphate were eluted at retention times around 1.72 min and 1.353 min respectively with symmetric peak shape. The data were collected and analyzed with software in a computer system. Diluent was made by addition of 0.3ml of Acetic acid with 5.0ml of Acetonitrile then it was made up to 100ml with water.

Preparations

Accurately weighed about 100mg of Glucosamine sulphate and 80mg of Chondroitin sulphate into a 100ml volumetric flask and dissolved with 25ml of diluent and sonicated for 5min and made up to volume with diluent. This solution was filtered through 0.45 µm filter paper. Twenty soflet formulations were weighed; gelatin was peeled out and finely powdered. An accurately weighed sample of powdered soflets equivalent to 2500mg of Glucosamine sulphate was extracted with diluent in a 40 ml volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper. The solution obtained was diluted with the diluent so as to obtain a concentration. All determinations were carried out in five injections. The contents of the mobile phase were filtered before use through 0.45µm filter paper, and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. Then, 20 µl of each of standard and sample solutions were injected into the HPLC system for six times to get the chromatograms. The retention time and average peak areas were recorded. A typical chromatogram of Glucosamine and Chondroitin Sulphate in formulation was shown in Figure 1. The amount of drug present in pharmaceutical formulation was calculated.

Validation

The described method has been validated for the assay of Glucosamine and Chondroitin Sulphate using following parameters [5,6,7,8]. The linearity range was found to be in between 80 to 120% concentrations of both drugs. The linearity range and linearity graphs were shown in Figures 2 and 3. To obtain proportionality, the slope of the regression line was calculated statistically by the method of “least squares” of area Vs concentrations for Glucosamine and Chondroitin sulphate. Then Correlation Coefficient was calculated. Precision was studied to find out variations in the test methods of Glucosamine (1000µg/ml) and Chondroitin Sulphate (800µg/ml) on the same day and on different day by using different make column of same dimensions (Ruggedness). The standard solution was injected for five times and measured the area for all five injections in HPLC. Precision and Ruggedness were done on the same day and the different day respectively and the %RSD was calculated for each. The accuracy of the method was shown by analyzing model mixtures which were obtained by spiking known amounts of Glucosamine sulphate and Chondroitin sulphate to the placebo. The model mixtures contained 80, 100 and 120% of Glucosamine and Chondroitin sulphate compared to the labeled drug amount. After injection, Accuracy -80%, Accuracy -100% and Accuracy -120% solutions, the Amount found and individual recovery values were calculated. Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to present in the sample matrix. It was found that the proposed method was specific as there is no interference of other active ingredients and excipients ensuring that the peak response is due only to the components. As part of the Robustness, deliberate change in the Flow rate and Mobile Phase composition were made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min.

RESULTS

A reverse – phase isocratic procedure is proposed as a suitable method for the analysis of Glucosamine and Chondroitin sulphate in soflets. A mixture of Octane sulphonic acid in water: Acetonitrile : triethylamine at the ratio of 90.65 : 8.96 : 0.381(adjusted the pH 4.0 with ortho phosphoric acid) at a flow rate of 1.0ml/min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte. The retention time was found to be 1.72 min and 1.353 min for Glucosamine and Chondroitin sulphate respectively. The percentage of purity of Glucosamine and Chondroitin sulphate in soflet dosage form is 99.24 and 91.67%. System suitability for the Glucosamine and Chondroitin sulphate, Theoretical Plates obtained from the standard injections was not less than 2000. Value of capacity factor of each elute was equal to 1 which produce the rapid elution and selectivity factor was approximately 2 which produces clear separation in minimal time. Resolution was 1.399, even our resolution results shown good efficiency and selectivity. As shown in the Figure 1, the substances were eluted forming well shaped, symmetrical single peaks,

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well removed from the solvent front. The precision of the HPLC system was determined using the %RSD of the peak areas for five injections of the standard solution of Glucosamine and Chondroitin sulphate. Precision data were present in Table 1. The %RSD was less than 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures of Glucosamine and Chondroitin sulphate. The recovery of Glucosamine and Chondroitin sulphate was evaluated from 80 to 120% of the labeled soflet. The mean percentage recoveries were found to be 99.1% and 100.8% for Glucosamine and Chondroitin sulphate individually. Accuracy data were present in Table 2. For quantitative application a linear calibration curve was obtained over the concentration range from 80 to 120% for Glucosamine and Chondroitin sulphate. Correlation coefficient for Glucosamine and Chondroitin sulphate were 0.9951 and 0.9954 respectively. Percentage curve fitting for Glucosamine and Chondroitin sulphate was found to be 99.51% and 99.54% respectively. The results of robustness indicate that the variation in flow rate affected the method significantly. The method is robust only in less flow condition. Even variation in organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±20% for Glucosamine and Chondroitin sulphate.

Table 1: Data for Precision

Standard solution	Precision		Ruggedness	
	Glucosamine sulphate	Chondroitin sulphate	Glucosamine sulphate	Chondroitin sulphate
Peak area	1616747	16589678	1688500	17223921
	1606811	16434890	1654331	17074800
	1599758	16473014	1652935	17101404
	1600475	16549535	1657894	17123724
	1599760	16591870	1656812	17083991
Mean	1604710.2	16527797.4	1662094	17121568
Standard deviation	7352.9518	70781.4168	14891	60180.25
%RSD	0.458	0.428	0.895	0.351

Table 2: Data for accuracy

Accuracy level (%)	Theoretical found in mg		Actual found in mg		% Recovery		%RSD	
	CS	GS	CS	GS	CS	GS	CS	GS
80%	0.5922	0.7959	0.6039	0.7896	102.0	99.2		
80%	0.5940	0.7989	0.6030	0.8022	100.5	100.4		
80%	0.5931	0.8019	0.6038	0.8175	100.8	100.2	0.247292	1.397302
100%	0.7370	0.9894	0.7439	0.9843	100.9	99.5		
100%	0.7343	0.9884	0.7437	0.9828	100.3	99.4		
100%	0.7361	0.9974	0.7437	0.9904	100.1	99.3	0.20597	0.100604
120%	0.8718	1.1859	0.8593	1.1656	99.3	99.3		
120%	0.8727	1.1829	0.8596	1.1669	99.6	99.6		
120%	0.8718	1.1859	0.8590	1.1756	99.1	99.1	0.255927	0.409607

Figure 1: A Typical Chromatogram of Glucosamine and Chondroitin sulphate

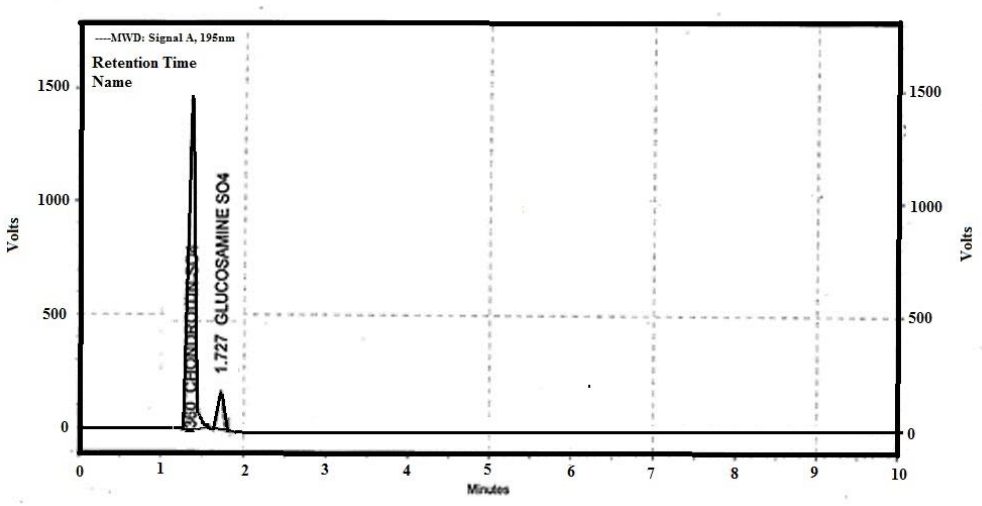

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Figure 2: Linearity curve for Glucosamine sulphate

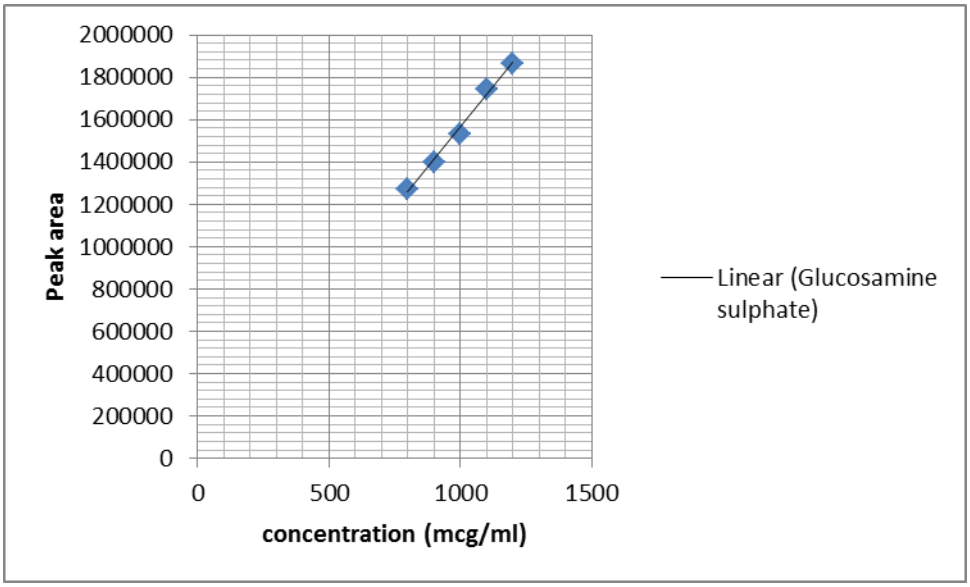
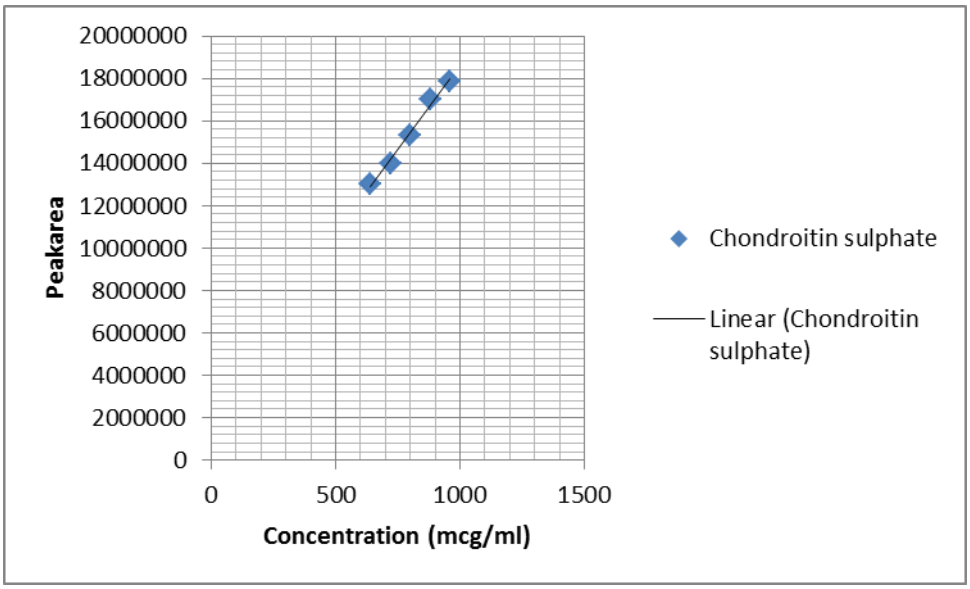


Figure 3: Linearity curve for Chondroitin sulphate



DISCUSSION

The developed method can be used for routine analysis because the linearity found in Glucosamine and Chondroitin sulphate is nearby to 1 that is not a single component lies below 0.9951 which shows the good regression for linearity. As system suitability parameters are concern the %RSD of each parameter lies below the limit of 2% as per the norms of ICH. Maximum recovery obtained by this developed method and the mean percentage recoveries for each component is nearby 100%. So, method can be used for the routine analysis and one most important reason is that the developed method [3,4] does not require use of expensive reagent and also less time consuming. The HPLC assay methods used in our study will not detect other compounds (impurity or related substances) that might be present in the product along with chondroitin sulfate or glucosamine. Most of the existing methods consumed expensive reagents for individual drug analysis or required more time for elution. But our proposed method requires less time for elution of chondroitin sulfate and glucosamine simultaneously.

CONCLUSION

The presented method is precise, sensitive and accurate. The advantages of proposed method are its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of mixtures of Glucosamine and Chondroitin sulphate in pharmaceuticals.

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