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Spectrophotometric determination of Chondroitin sulfate in bulk drug and pharmaceutical formulation

Somashekar P.L[1*], Tripathy A. S[1], Chandrashekar Javali[1], Sathish K.P[2], Palakshi Gouda O[3]

[1]Govt College of Pharmacy, Bangalore, Karnataka, India
[2]J S S College of Pharmacy, Mysore, Karnataka, India
[3]V L College of Pharmacy, Raichur, Karnataka, India

ABSTRACT

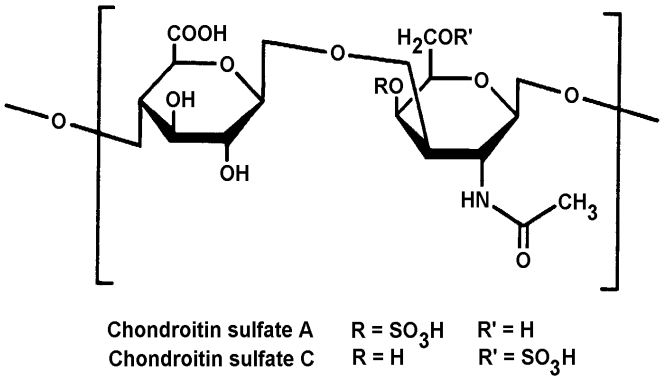
Simple, specific, precise and accurate spectrophotometric method has been developed for the estimation of chondroitin sulfate in bulk and tablet dosage form. The proposed method is based on the principle that chondroitin sulfate condensed with carbazole in presence of strong acid gave pink colour indication at wavelength of maximum absorbance at 530 nm. The Beer-Lambert's law obeyed in the range of 40 – 120 µg/ml with correlation coefficient was at 0.999. The results presented are statistically validated in accordance with the guidelines provided by ICH. The recovery studies were carried out at three different levels. The precision was good with RSD lower than 2.0 %. The developed method was considerably easy, simple, reproducible and cost effective.

Keywords: Spectrophotometric method, Chondroitin sulfate, Carbazole.

INTRODUCTION

Chondroitin sulfate (CHS) belongs to a family of heteropolysaccharides called Glycosaminoglycans (GAGs). GAGs are large complexes of negatively charged heteropolysaccharides chains generally associated with small amount of protein, which are formerly known as mucopolysaccharides[1,2]. GAGs in the form of Proteoglycans comprise the ground substance in the extra cellular matrix of connective tissue. CHS is chemically Poly- (1-3)-N-acetyl-2-amino-2-deoxy-3-O-ß-D-glucopyranurosyl – 4 - (or 6-) sulfonyl-D-galactose. CHS is made up of linear repeating units containing D-galactosamine and D-glucuronic acid. The amino group of galactosamines in the basic unit of CHS is acetylated, yielding N-acetyl-galactosamine. The sulfate group is esterified to the carbon 4 or 6 position in N-acetyl-galactosamine yield Chondroitin sulfate A and Chondroitin sulfate C respectively. The molecular weight of chondroitin sulfate A or C ranges from 5,000 to 50,000 Daltons and

contains about 15 to 150 basic units of D-galactosamine and D-glucuronic acid[3]. It is represented by the following structural formula:



Chondroitin sulfate A R = SO_3H R' = H
Chondroitin sulfate C R = H R' = SO_3H

Figure 1- Chemical structure of Chondroitin sulfate

CHS mainly used in promotion and maintenance of the structure and function of cartilage, pain relief of osteoarthritic joints and anti-inflammatory activity

Literature review revealed that few analytical methods include Gel-exclusion chromatography[4,5], capillary electrophoresis[6] and titration with cetyl pyridinium chloride detecting end point with a phototrode[7,8]. The other methods in the literature for quantitating chondroitin sulfate needs enzymatic digestion followed by disaccharide analyses, include reverse phase high performance liquid chromatography[9] (HPLC), anion-exchange chromatography[10] and gel electrophoreses with fluorescence detection[11].

Hence an attempt has been made to develop accurate, precise and reproducible colorimetric method for estimation of chondroitin sulfate in bulk and tablet formulation. The method was validated by using various parameters as per ICH guidelines[12].

MATERIALS AND METHODS

Instrumentation

Perkin Elmer double beam UV-Visible spectrophotometer was used with 1 cm matched quartz cells.

Chemicals and Reagents

Pure chondroitin sulfate was obtained as gift sample from Banner Pharmacaps (India) Pvt Ltd, Bangalore, India, carbazole (Rolex AR Grade), sodium tetraborate decahydrate (Merck), concentrated sulfuric acid (Qualigens fine chemicals) and double distilled water were used.

Reagent: 1.Carbazole reagent (0.1%w/v in ethanol)
2. Sodium tetraborate decahydrate (0.2% w/v in concentrated sulfuric acid)

Preparation of Standard Stock Solution

About 100 mg of chondroitin sulfate working reference standard of known purity (92.42% pure) was accurately weighed into a 100 ml volumetric flask, dissolved & volume was made upto 100 ml with water (1mg/ml).

Determination of Wavelength of Maximum Absorbance

2 ml standard stock solution was transferred into 25 ml volumetric flasks, 10 ml of 0.2% sodium tetraborate decahydrate solution was added and solution was cooled to room temperature. The solution mixture was heated in a boiling water bath for 20 minutes and cooled to room temperature. Then 2 ml of 0.1% carbazole solution was added, again heated on the boiling water bath for 20 minutes and cooled to room temperature. The volume was made upto 25 ml distilled water. This pink coloured solution was scanned in the 400 – 800 nm range against reagent blank. The wavelength of maximum absorbance of pink colour chromogen was found at 530 nm as shown in fig no.2

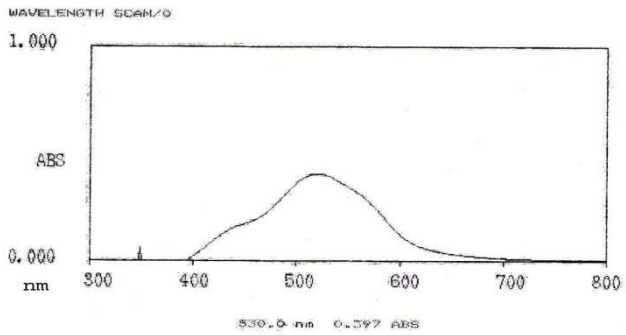
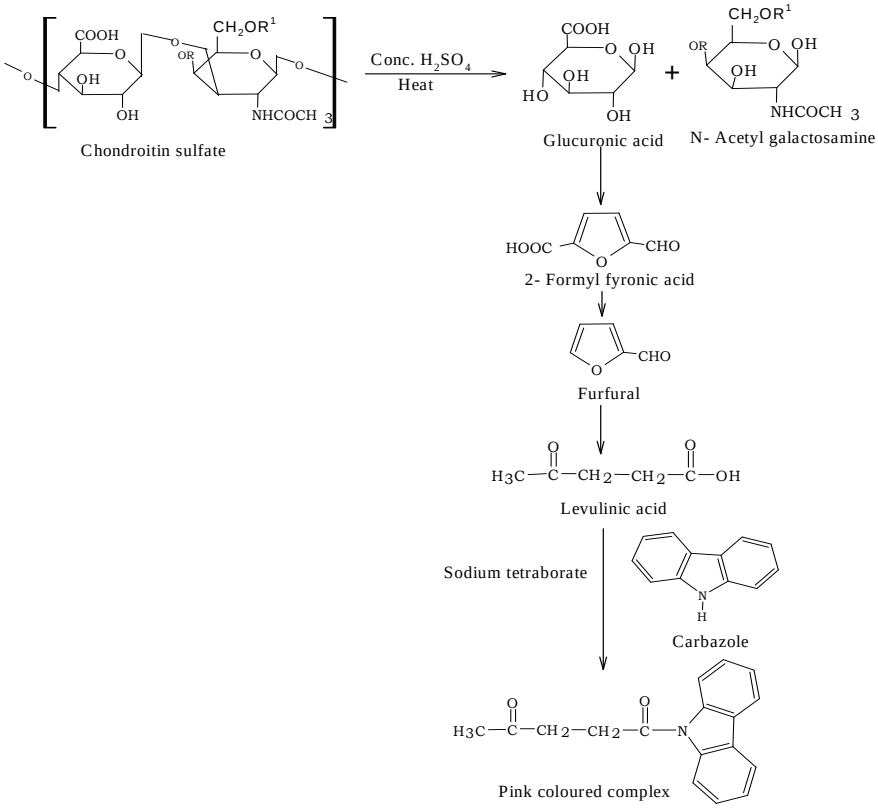


Figure 2 - Absorbance maxima of Chondroitin sulfate

Reaction scheme for Chondroitin sulfate with Carbazole



Assay of Marketed Formulation

Twenty tablets were accurately weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 100mg of chondroitin sulfate was transferred to 100ml volumetric flask and sonicated for 15 minutes in about 50 ml of double distilled water and

made up the volume with double distilled water. The resulting solution was filtered through Whatman filter paper. 2 ml of filtrate was transferred into 25 ml volumetric flask, 10 ml of 0.2% sodium tetraborate decahydrate solution was added and solution was cooled to room temperature. The solution mixture was heated in a boiling water bath for 20 minutes and cooled to room temperature. Then 2 ml of 0.1% carbazole solution was added, again heated on the boiling water bath for 20 minutes and cooled to room temperature. The absorbance of pink colour chromogen was measured at 530 nm against reagent blank. The amount of chondroitin sulfate was calculated using following formula.

Calculation:

$$\frac{A_{test}}{A_{std}} X \frac{Wt_{.std}}{100} X \frac{2}{25} X \frac{100}{Wt_{.test}} X \frac{25}{2} X \frac{P}{100} X \quad W_{Avg}$$

Where,

A_{test} = Absorbance of the chondroitin sulfate in the sample.
A_{std} = Absorbance of the chondroitin sulfate in the standard
$Wt._{std}$ = Weight of the chondroitin sulfate working reference standard in mg
$Wt._{test}$ = Weight of the sample taken in mg
P = Percent purity of chondroitin sulfate working reference standard (92.42 %)
W_{Avg} = Average Weight of the tablet in mg

Method Validation

Validation is the process of established documented evidence which provides high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. The method was validated by various parameters as per ICH guidelines (table no 2)

Linearity

Varying concentrations of chondroitin sulfate were treated with carbazole within the range 40 µg/ml to 120 µg/ml corresponding to 50%, 75%, 100%, 125%, and 150% of chondroitin sulfate. The linearity of chondroitin sulfate was found to be 40 -120 µg/ml and linear regression was found to be $r^2 = 0.9998$.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard chondroitin sulfate at three different concentrations (50%, 100%, and 150%). Result of the recovery study were found to be within the acceptable criteria 100 ± 10 %, indicates sensitivity of the method towards detection of chondroitin sulfate and non interference of excipients in the method (table no1).

System Precision

The precision of the system was determined by 6 repetitive absorbance of the same standard solutions by using 2 ml of stock solution. As the value of % RSD of system precision study were in within the acceptable limit (less than 2%). Hence the method provides good precision.

Method Precision

The precision of the method for the assay of chondroitin sulfate was determined by the assay of six aliquots of the homogeneous sample. As the value of % RSD of system precision study were in within the acceptable limit (less than 2%). Hence the method provides good precision and reproducibility.

Table No –1: Recovery Calculation for chondroitin sulfate

Spike level	Theoretical Value of Chondroitin sulfate (mg/ml)	Practical Value of Chondroitin sulfate (mg/ml)	Recovery (%)	Average	RSD (%)
Level-I				99.09	1.28
50 % -1	0.504	0.4971	98.63		
50 % -2	0.502	0.5047	100.53		
50 % -3	0.504	0.4945	98.11		
Level-II				99.32	0.82
100 % -1	1.012	0.9967	98.48		
100 % -2	1.008	1.0018	99.38		
100 % -3	1.026	1.0272	100.11		
Level-III				100.07	0.83
150 % -1	1.497	1.4837	99.11		
150 % -2	1.502	1.5091	100.47		
150 % -3	1.507	1.5167	100.64		

Specificity

The study was conducted to prove that the absorbance obtained in the samples is only due to chondroitin sulfate without any interference from other excipients. Placebo solution at varying concentration does not show any absorbance at 530 nm. Hence the method is specific for the determination of chondroitin sulfate.

Solution Stability

The stability of the analytical solution for assay of chondroitin sulfate was determined by the assay of sample preparation at fixed intervals of time. The % RSD for the assay values for chondroitin sulfate up to 24 hours is 0.74. This indicates that the analytical solution is stable up to 24 hours.

Ruggedness

The ruggedness of the method for assay of chondroitin sulfate was determined by the assay of six aliquots of the homogeneous sample on different instrument and by different analyst. The % RSD for the assay values for chondroitin sulfate in ruggedness study was found to be 0.26. This indicates that the method has good reproducibility and very less random error. The results of the method developed and validated for chondroitin sulfate in formulation are depicted in the table no 2. The results developed method showed good agreement with the labeled claim in the formulation analyzed.

Table No- 2 : Summarized tabulated results for validation of the analytical method for chondroitin sulfate

Parameter	Acceptance criteria	Results Obtained
1. Linearity	Regression coefficient (r^2) not less than 0.999 Beer's Range Regression Equation	0.9998 40 – 120 µg/ml y = 0.0051 x+0.0017
2. Accuracy	Recovery between 98 – 102 %	99.08 -100.7 %
3.System precision	% RSD should be less than 2.0 %	1.36 %
4. Method precision	% RSD should be less than 2.0 %	1.22 %
5. Specificity	Non interference of placebo and blank in analysis	Complies
6. Ruggedness	% RSD should be less than 2.0 %	0.261 %
7.Sandell's sensitivity (mg/ml/0.001 abs units)	-	0.0215

RESULTS AND DISCUSSION

The proposed method for the estimation of chondroitin sulfate is based on the reaction between carbazole and hydrolyzed component of chondroitin sulfate. The chondroitin sulfate hydrolyzed by concentrated sulfuric acid gave glucuronic acid, which upon condensation with carbazole in presence of sodium tetraborate gave pink colour. The solution has absorption maxima at 530 nm and obeyed Beer's concentration range 20 – 120 µg/ml. The accuracy of the method was determined at 50%, 100% and 150% level and the % recovery ranged from 99.08 - 100.7 %. The % RSD less than 2% indicates the method was accurate and precise. The method was found to be sensitive with respect to Sandell's sensitivity.

Hence developed method was found to be simple, accurate, precise, reliable and reproducible for routine quantitative estimation of chondroitin sulfate, which can be adopted in Quality control laboratories.

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