ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 245–249

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Development and validation of a photometric titration method for the quantitation of sodium chondroitin sulfate (bovine) in Cosequin® DS chewable tablet

Zhongming Liang *, Corrine Bonneville, Terrin Senez, Todd Henderson

Nutramax Laboratories®, Inc., 2208 Lakeside Blvd., Edgewood, MD 21040, USA

Received 26 January 2001; received in revised form 5 September 2001; accepted 26 September 2001

Abstract

A photometric titration method was developed and validated to quantitate sodium chondroitin sulfate in raw materials and Cosequin® DS chewable tablet [1]. About 0.1% (w/v) cetylpyridinium chloride was used to titrate sodium chondroitin sulfate with photometric indication at wavelengh 420 nm. The standard curves for sodium chondroitin sulfate showed linearity ($r \geq 0.99$) over the selected concentration range from 0.6 to 1.4 mg/ml. The chewable tablet was ground to fine powder and extracted with water and the resulting solutions filtered through a 0.45 μm membrane filter. Recovery between 97 and 103%,. The intra- and inter- day precision as indicated by the relative standard deviation (R.S.D.) were not greater than 0.33 and 0.78%, respectively. The method was found to be specific and with excellent linearity, accuracy and precision and is well suited for the quantitation of sodium chondroitin sulfate in raw material and Cosequin® DS chewable tablet. © 2001 Published by Elsevier Science B.V.

Keywords: Sodium chondroitin sulfate; Nutraceutical; Titration; Quantitation

1. Introduction

Chondroitin sulfate is a glycosaminoglycan that makes up a large proportion of cartilage components. It is a heteropolysaccaride made up of glucuronic acid and galactosamine. Clinical studies have shown that if administered orally in a pure form of approximately 16 900 MW this molecule is bioavailable [1,2] and efficacious in both the treatment [3–5] and prevention [6,7] of osteoarthritis. Other molecular weights have not been shown to be as permeable in a CaCo-2, [8] or in vivo [9] models of absorption.

Recently there has been a flood of dietary supplements containing glucosamine and chondroitin sulfate into the marketplace [10]. Some brands have been shown to be clinically effective in reducing pain [11–14], as well as stabilizing articular cartilage [15,16]. Of concern is the documented lack of quality in many of the marketed products

* Corrosponding author. Tel.: +1-410-776-4000; fax: +1-410-776-4009.

E-mail address: zhongmingliang@hotmail.com (Z. Liang).

[1] Cosequin® DS chewable tablet contains glucosamine HCl, sodium chondroitin sulfate, manganese ascorbate, and flavoring agents.

0731-7085/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0731-7085(01)00563-5

both in finished dosage forms as well as raw materials [10].

While there exists validated assay methods for a simple powder formulation [17], these methods are not optimal for more complex dosage forms. Several other methods for separating and quantitating chondroitin sulfate and other GAG's need digesting the polysaccharide into disaccharide units. The disaccharide then separated using reverse-phase high performance liquid chromatography (HPLC) [18], anion-exchange resin [19] capillary electrophoresis [20] with UV spectroscopic, fluorescence detection. The purpose of this work was to develop a precise and accurate phototrode method that could be applied to the quantitation of chondroitin sulfate in a specific complex chewable tablet formulation.

2. Experimental

2.1. Materials and reagents

Chondroitin sulfate sodium (from bovine trachea) was purchased from Bioiberica (Poligono Industrial, Barcelona, Spain). The structure of chondroitin sulfate is shown in Fig. 1. Cetylpyridinium chloride was purchased from Acros Organics (New Jersey). Potassium dihydrogen phosphate (KH_2PO_4 HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ), Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) was purchased from Sigma (St. Louis, MO). Xantural 180 (lot100405 or equivalent). All chemicals and solvents were ACS analytical grade or HPLC grade. Deionized water was prepared by an ultrapure water system from Chesapeake Instruments Inc (Columbia, MD). Cosequin® DS chewable tablet (Nutramax Laboratories® Inc).

2.2. Standard and sample preparation for raw materials and finished products

Chondroitin Sulfate standard curve sample was prepared in water in 100 ml volumetric flask, the concentration range from 0.6 to 1.4 mg/ml. A stock solution was prepared at a concentration of 2 mg/ml and serial dilutions were made to give concentration of standards of 0.6, 0.8, 1.0, 1.2, 1.4 mg/ml. To each standard 10 ml of a pH 7.0 phosphate buffer were added. Sonicate or shake 10 min. Pipet 5.0 ml aliquots into 50 ml beaker, add 25 ml of water, stir bar and start titration. For Cosequin® DS chewable tablets assay, weigh 100 mg chondroitin sulfate standard and 21.8 mg Xantural 180 into 100 ml volumetric flask, add 10 ml of pH 7.0 phosphate buffer. Sonicate or shake 10 min. Pipet 5.0 ml aliquots into 50 ml beaker, add 25 ml of water, stir bar and start titration.

2.3. Preparation of test solution

Weigh accurately ten Cosequin® DS chewable tablets individually and record the weight, ground the tablets to fine powder, Weigh accurately the equivalent of 100 mg chondroitin sulfate into a 100 ml volumetric flask dissolve in 50 ml water, add 10 ml of phosphate buffer, sonicate or shake 10 min and dilute to volume with water. Filter a portion of the solution (about 20 ml) with 0.45 μm filter. Pipet 5.0 ml aliquots into 50 ml beaker, add 25 ml of water, stir bar and start titration.

2.4. Instrumentation

The sample were analyzed by an Titrino, 751 GPD (Brinkmann Instruments Inc, Westbury, NY 11590) with Colorimeter, Brinkmann 910 (wavelength 420 nm, Brinkmann Instruments Inc). The titrino was coupled with an Brinkmann Titrino Workcell Version 4.3 (Brinkmann Instruments Inc). The sample was titrated by 0.1% cetylpyridiniun chloride.

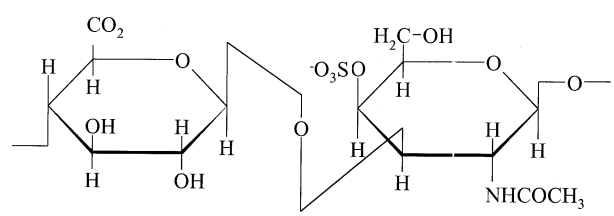

Fig. 1. Structure of chondroitin sulfate.

2.5. Assay validation

2.5.1. Linearity and range

Linearity of the chondroitin sulfate assay was determined by analysis of four replicates of five concentrations (0.6, 0.8, 1.0, 1.2, 1.4 mg/ml) by least squares regression. The minimum acceptable coefficient to establish linearity was set at 0.999 a priori.

2.5.2. Method precision and percent recovery

Precision of the assay was established by analysis of four replicate ($n = 4$) of a standard solution of the analyte. To determine intra-day precision of the assay, replicate ($n = 4$) standard solution of the analyte at five different concentrations (0.6, 0.8, 1.0, 1.2, 1.4 mg/ml) were analyzed at the same day. To determine inter-day precision of the assay, replicate ($n = 4$) standard were analyzed on three different days. The percent relative standard deviation (R.S.D.) of the assay results were determined.

Extraction efficiency was determined by spiking chondroitin sulfate into Cosequin® DS chewable tablet in the following concentration 0.2, 0.4, 0.6 mg/ml. The percent recovery was determined by the following equation:

$$\%\text{Recovery} = \frac{\text{Amount(total)} - \text{Amount(chewable tablet sample)}}{\text{Amount added}} \times 100$$

2.5.3. Accuracy

Accuracy of the method was determined by calculating the mean concentration of three replicate ($n = 3$) solutions at concentrations ranging from 0.6 to 1.4 mg/ml. The standard in the concentration range of 0.6–1.4 mg/ml from three different runs performed over several days were used to check for accuracy. The means of the three runs were calculated and compared with the spiked value to determine the percentage difference between the mean and the spiked value (amount added). The percentage relative error was determined as:

$$\%\text{Relative error (R.E.)} = \frac{[\text{mean} - \text{spiked}]}{[\text{spiked}]} \times 100$$

Between and within run accuracy were also determined.

2.5.4. Specificity, stability and robustness

Specificity was evaluated by testing samples to ensure that no interference from excipients. Stability of the analyte in standards and chewable tablet at room temperature were determined. This was performed by analyzing the standard solutions in triplicate at different times after storage at room temperature. Robustness was assessed by determining the chondroitin sulfate at different pH, temperature, buffer solution and different amount of water in the titration solution.

2.6. Ruggedness

The ruggedness was assessed by determining the chondroitin sulfate using two different batches of titration reagent and two chemists running the same range standard curve.

2.7. Analysis of chewable tablet

Weigh accurately ten tablets individually and record the weight, ground the tablets to fine powder, weigh 428 mg of the powder into a 100 ml volumetric flask dissolve in 50 ml water, add 10 ml of phosphate buffer, sonicate 10 min and dilute to volume with water. Filter a portion of the solution (about 20 ml) with 0.45 μm filter. Pipet 5.0 ml aliquots into 50 ml beaker, add 25 ml of water, stir bar and start titration. Titrate twice for each sample and report the mean of two. The percent of the label claim for the Cosequin® DS chewable tablets was calculated as follows:

$$\%\ \text{Label claim} = \frac{\text{Assayed amount (mg)}}{\text{labeled amount (mg)}} \times\ 100$$

3. Results

3.1. Validation assay precision

The amount of the chondroitin sulfate in chewable tablet was calculated by comparing the end point with the standard. The standard curves for

Table 1
Intra-day and inter-day assay precision for chondroitin sulfate

Concentration (mg/ml)	Intra-day (% R.S.D.)	Inter-day (% R.S.D.)
0.2	0.08	1.00
0.4	0.42	0.11
0.6	0.63	1.22
0.8	0.21	0.54

chondroitin sulfate showed linearity over the selected concentration range from 0.6 to 1.2 mg/ml for chewable tablet with consistent slopes and excellent correlation coefficients ($r \geq 0.999$) throughout the validation runs. Method precision was determined by analyzing ten replicate samples of Cosequin® DS chewable tablets according to the method. Results ranged from 379.62 per tablet to 409.17 mg per tablet with an average 392.26 per tablet with a 2.26% R.S.D. The intra-day and inter-day precision data for chondroitin sulfate in Cosequin® DS chewable tablet are listed in Table 1. The intra-day and inter-day% R.S.D. ranged from 0.14 to 0.69%, respectively. Table 2 summarizes the intra-day and inter-day accuracy date for chondroitin sulfate in Cosequin® DS chewable tablet. The intra-day and inter-day accuracy, as indicated by R.E., ranged from 5.13 to 1.28%, respectively. After the comparison of the amount spiked into chewable tablet, the extraction recovery was found to be 99.75, 97.09, 97.56 for the 0.2, 0.4, 0.6 mg/ml standard added to the chewable tablet, respectively.

Table 2
Intra-day and inter-day assay accuracy for chondroitin sulfate

Concentration (mg/ml)	Intra-day (% R.E.)	Inter-day (% R.E.)
0.2	−0.02	−0.25
0.4	−0.29	−0.08
0.6	−1.00	−1.88
0.8	−0.92	−2.1

3.2. Stability

Stability of chondroitin sulfate was evaluated by comparing the amount of chondroitin sulfate in chewable tablet that had been in storage at room temperature with the amount of a freshly prepared standard. The chondroitin sulfate was stable after 45 h when stored at room temperature (25 °C).

3.3. Specificity

All the excipients were analyzed by the method to determine if these components interfered with the chondroitin sulfate assay. The excipient xantural 180 in Cosequin® DS chewable tablet has interference for the assay of chondroitin sulfate in Cosequin® DS chewable tablet. The interference can be deducted by adding the same amount of xantural 180 in chondroitin sulfate standard as the amount of xantural 180 in the chewable tablet and establish very good precision and recovery.

3.4. Chewable tablet assay and content uniformity

The assay was done by analyzing ten samples from ten different lots (see Table 3). Results ranged from 395.55 per tablet to 409.27 mg per tablet with an average of 401.05 mg per tablet and a 1.20% R.S.D.

3.5. Robustness

The titration conditions were evaluated by determining the chondroitin sulfate at pH 5, 6, 7, 8, 9. Temperature at 15, 20, 25, 30, 35 °C. Buffer solution (pH 7) in 8, 9, 10, 11, 12 ml. Amount of water for titration in 20, 22, 25, 27, 30 ml. The %R.S.D. for chondroitin sulfate assay are 0.54, 0.18, 0.33 and 1.39, respectively.

3.6. Ruggedness

Two different batches of titration reagent and two chemists running the same range standard curve with consistent slopes and excellent correlation coefficients ($r > 0.999$).

Table 3
Assay ten different lots of chondroitin sulfate chewable tablet

Sample	Label content (mg)	CS amount (mg per tablet)	% Label claim
1	400	407.70	107.29
2	400	400.55	105.41
3	400	398.92	104.98
4	400	401.71	105.71
5	400	400.83	105.48
6	400	394.27	103.76
7	400	395.55	104.09
8	400	399.73	105.19
9	400	409.27	107.70
10	400	400.05	105.28

4. Discussion

Most titration methods [21–23] has focused on analysis of disaccharide units after enzymatic breakdown of the chondroitin sulfate. Other methods in the literature for chondroitin sulfate use pre-column preparation, including enzymatic digestion and acid digestion. Pre- and post column derivitization for fluorescence and chemiluminescence detection. These methods can be difficult and time consuming. The photometric titration method we developed using automatic instrument (Brinkmann Titrino with Colorimeter) and analyze chondroitin sulfate directly instead of analyze disaccharide after enzymatic digestion, the advantages using photometric titrition are fast and accurate, each sample take 4–5 min, the reagent (Cetylpyridinium) bind with chondroitin sulphate group and form a complex compound and precipitated out, using the colorimeter to measure the biggest response occurred in the reaction and get the end point. This method is fast, specific and precise. It has been successfully used in quantitation of chondroitin sulfate as a raw material and in Consequin® chewable tablet.

References

[1] A. Conte, N. Volpi, L. Palmiera, I. Bahous, G. Ronca, Drug Res. 45 (1995) 918–925.
[2] N. Volpi, M. Roth, D. Uebelhart, Presented at XIV European League Against Rheumatism Congress, Scotland England, 6–11 June, 1999.
[3] C.L. Deal, R.W. Moskowitz, Rheum. Dis. Clin. North Am. 25 (2) (1999) 379–395.
[4] F. Barkhard, L.H. Schwitzer, K. Montag, J. Rheumatol. 27 (1) (2000) 205–211.
[5] T.E. McAlindon, J. Culin, D.T. Felson, Arthritis Rheum. 41 (1998) S198.
[6] D. Uebelhart, E.J.M.A. Thonar, P.D. Delmus, et al., Osteoarthritis Cartilage Suppl. A (6) (1998) 39–46.
[7] G. Verbruggen, S. Goemaere, E.M. Veys, Osteoarthritis Cartilage Suppl. A (6) (1998) 37–38.
[8] A. Adebowale, D. Cox, Z. Liang, N.D. Eddington, J. Am. Nutraceutical Assoc. 3 (1) (2000) 37–44.
[9] S. Yamanashi, H. Toyoda, N. Furuya, T. Harada, K. Yoshida, T. Toida, T. Imanari, Yakugaku Zasshi 111 (1) (1991) 73–76.
[10] A. Adebowale, Z. Laing, N. Eddington, Nutraceuticals, J. Nutraceuticals Funct. Med. Goods 2 (2) (1999) 15–29.
[11] A.K. Das, T.A. Hammad, Osteoarthritis Cartilage 8 (5) (2000) 343–350.
[12] C.T. Leffler, A.F. Philippi, S.G. Leffler, J.C. Mosure, P.D. Kim, Mil. Med. 164 (2) (1999) 85–91.
[13] S.O. Canapp, R.M. McLaughlin, J.J. Hoskinson, J.K. Roush, M.D. Butine, Am. J. Vet. Res. 60 (12) (1999) 1550–1556.
[14] M.A. Anderson, M.R. Slater, T.A. Hammad, Prev. Vet. Med. 38 (1999) 65–73.
[15] L. Lippiello, J. Woodward, R. Karpman, T. Hammad, September 1999, Accepted for publication in Clin. Orthop. Related Res.
[16] J. Beren, S. Hill, T. Hammad, N. Rose, Accepted for publication in Proc. Soc. Exp. Biol. Med., 2000.
[17] A. Adebowale, Z. Liang, M. Ashraf, N.D. Eddington, Accepted for publication in the J. Pharma. Biomed. Anal.
[18] N. Karamanos, A. Syrokou, P. Vanky, M. Nurminen, A. Hjerpe, Anal. Biochem. 221 (1994) 189–199.
[19] A. Li-Hakim, R. Linhardt, Anal. Biochem. 195 (1991) 68–73.
[20] W. Way, K. Gibson, A. Breite, J. Liq. Chrom. Rel. Technol., 23 (18) (2000).
[21] J. Okazaki, K. Kakudo, A. Kamada, E. Utoh, Y. Gonda, Eur. J. Oral Sci. 105 (5 Pt 1) (1997) 440–443.
[22] J. Okazaki, A. Kamada, Y. Gonda, T. Sakaki, G. Embery, Oral Dis. 2 (3) (1996) 224–227.
[23] G. Kahaly, C. Hansen, J. Byer, R. Winand, J. Endocrinol. Invest. 17 (1) (1994) 45–50.