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Quantitative analysis of chondroitin sulfate in raw materials, ophthalmic solutions, soft capsules and liquid preparations

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Abstract

We performed the quantitative analysis of chondroitin sulfate (CS) obtained from raw materials and various pharmaceutical preparations. To quantify CS content in raw materials and in an ophthalmic solution, each test sample and the authentic CS were first digested by chondroitinase ABC. The CS disaccharides produced were analyzed by strong anion-exchange high-performance liquid chromatography (SAX-HPLC) and CS content was quantified by calculating the total peak areas of the disaccharides derived from a CS calibration curve. In the case of soft capsules, CS was first extracted with hexane followed by phenol–chloroform to remove oil and protein ingredients. The extracted CS samples were depolymerized by chondroitinase ABC and CS content was determined. Quantitative analysis of the disaccharides derived from raw materials and an ophthalmic solution showed the CS contents (%) were 39.5 ± 0.1 to 105.6 ± 0.1 and 103.3 ± 1.2, respectively. In case of CS analysis in soft capsules and liquid preparations, the overall recovery (%) of the spiked CS was 96.79 ± 0.53–103.54 ± 1.78 and 97.10 ± 1.82 to 103.17 ± 2.34, respectively. In conclusion, the quantitative analysis of the disaccharides produced by enzymatic digestion can be used in the direct quantitation of CS containing pharmaceutical formulations.

Keywords: Chondroitin sulfate; Chondroitinase ABC; Formulation material; Ophthalmic solutions; Soft capsules; Liquid preparations

1. Introduction

Chondroitin sulfate (CS) is composed of an alternating sequence of sulfated and/or unsulfated D-glucuronic acid (GlcA) and *N*-acetyl-D-galactosamine (GalNAc) residues linked through alternating (β (1→3)) and (β (1→4)) bonds (Fig. 1). CS belongs to a heterogeneous family of glycosaminoglycans, since disaccharides having a different number and position of sulfate groups are located at various locations in the polysaccharide chains [1]. The component ratio of the CS disaccharides with non-sulfate, mono-sulfate, di-sulfate, and/or tri-sulfate is dependent on the species and/or tissues of origin [2]. According to previous results, CS originating from land animals (bovine and porcine) has more ΔDi-4S than ΔDi-6S in their structure, while CS originating from shark cartilage shows the opposite distribution [1,3].

CS, an essential component of extracellular matrix of connective tissues that plays an important role in the elasticity and function of the articular cartilage, is mainly attached covalently to core proteins in the form of proteoglycans [4]. It has been used clinically for the treatment of chronic diseases such as degenerative arthritis, cirrhosis, and chronic photo damage

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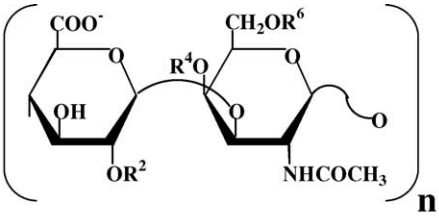

	R^2	R^4	R^6
△ Di-0S	H	H	H
△ Di-6S	H	H	SO_3^-
△ Di-4S	H	SO_3^-	H
△ Di-2,6 di S	SO_3^-	H	SO_3^-
△ Di-4,6 di S	H	SO_3^-	SO_3^-
△ Di-2,4 di S	SO_3^-	SO_3^-	H
△ Di-2,4,6 tri S	SO_3^-	SO_3^-	SO_3^-

Fig. 1. Structure of CS disaccharides and compositional properties.

[5–7]. Although the intestinal absorption of CS is controversial due to its high molecular weight (MW) and charge density [8,9], several investigations have demonstrated the efficacy and bioavailability of CS in animals [6,10–12] and humans [13–17]. Hence, the number of pharmaceuticals containing CS has increased as the types of formulations, which include tablets, hard capsules, soft capsules, ophthalmic solutions, and liquid preparations. To quantify CS in biological samples and/or pharmaceuticals, several analytical methods have been developed including the carbazole assay [18], dye binding analysis [19–21], agarose gel electrophoresis [14,22], enzymatic digestion using chondroitinase ABC [23,24], photometric titration [25], high performance size exclusion chromatography (HPSEC) [26], and in-capillary enzymatic reactions [27]. Among these, carbazole and dye binding methods are most commonly used for the quality control of CS as a pharmaceutical ingredient. However, these methods often cannot be used when impurities such as other carbohydrates, anionic molecules, and/or metals were present in a pharmaceutical formulation containing CS, as these result in interference in the UV absorbance [18,26]. Some methods have been applied for the quantitation of CS in hard capsules [26], tablets [25,26], and ophthalmic solutions [27], but the quantitative analysis of CS in soft capsules and liquid preparations has not been reported. The CS analysis of these pharmaceutical formulations is limited because many other ingredients (various vitamins and γ-oryzanol, etc.) mixed with CS and capsule materials (gelatin, glycerin, D-sorbitol, etc.) interfere with direct analysis. Furthermore, pretreatment to purify CS from other ingredients in a pharmaceutical formulation is invariably required, because various herb extracts, vitamins, and nutrition supplements are often included within liquid preparations.

The first purpose of the present study is to evaluate the quality of CS raw materials (CSRMs) being used for manufacturing CS containing pharmaceuticals. CSRMs are treated with chondroitinase ABC and the disaccharides are quantitatively analyzed by strong anion-exchange high-performance liquid chromatography (SAX-HPLC). In addition, the source and the molecular weight of CSRMs are also investigated. The second is to quantify the CS content in an ophthalmic solution, soft capsules, and liquid preparations. To remove the ingredients that interfere with the CS analysis in pharmaceuticals, the pretreatment method uses hexane/phenol-chloroform or phenol-chloroform to purify CS from soft capsules and liquid preparations, respectively.

2. Experimental

2.1. Chemicals

CSRMs were obtained from several local suppliers and pharmaceutical companies. Authentic CS, originating from bovine trachea, was purchased from New Zealand Pharmaceuticals (NZP; Palmerston North, New Zealand) and was utilized as a standard in these analyses. Two CSs (MW 15,000 and 40,000 Da) used as MW standards were obtained from Seikagaku (Tokyo, Japan) and acharan sulfate oligosaccharides (10-mer, 20-mer, and 30-mer) were purified by controlled enzymatic digestion as previously described [28]. Chondroitinase ABC from *Proteus vulgaris*, unsaturated CS disaccharides (ΔUA-[1→3]-GalNAc: ΔDi-0S, ΔUA-[1→3]-GalNAc-6S: ΔDi-6S, ΔUA-[1→3]-GalNAc-4S: ΔDi-4S, and ΔUA-2S-[1→3]-GalNAc-6S: ΔDi-2,6diS), boric acid, tris[hydroxymethyl]aminomethane (Trizma® base), sodium chloride, sodium acetate, ethylenediaminetetraacetic acid (EDTA), azure A, and phenol–chloroform–isoamyl alcohol (25:24:1) were purchased from Sigma (St Louis, MO, USA). Agarose was from Cambrex Bio Science (Rockland, ME, USA) and other reagents were of the best grade available.

2.2. HPLC analysis

HPLC analyses were performed on the ÄKTA purifier system (Amersham Pharmacia, Uppsala, Sweden) equipped with a P-900 pump, a UV detector, and a fraction collector. UNICORN software Version 3.1 (Amersham Pharmacia) was used to control apparatus and to collect data. SAX-HPLC was performed with a Hypersil SAX column (4.6 mm × 250 mm, 5 μm) from Thermo Hypersil-Keystone (Belefonte, PA, USA). After sample injection, the column was washed with water (adjusted to pH 3.5 with 1N hydrochloric acid) for 4.155 min corresponding to one column volume (CV). Then, a linear gradient of 0–1.0 M NaCl (pH 3.5) for 41.55 min (10 CV) was used and the profile was monitored at 232 nm over a period of 30 min during which all the disaccharides eluted. HPSEC was accomplished with a TSK G3000 SW column (7.5 mm × 300 mm) and a TSK guard column (7.5 mm × 75 mm) from Tosoh (Tokyo, Japan). The mobile phase was 0.1 M NaCl and UV absorption was mea-

sured at 210 nm. The mobile phases used for HPLC analyses were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and degassed under vacuum before use. The flow rate was 1.0 mL/min and the system was operated at ambient temperature.

2.3. Enzymatic digestion of CSRMs and CS in an ophthalmic solution

Authentic CS and CSRMs were dried at 105 °C for 4 h and dissolved with water to make a stock solution (10 mg/mL). Further dilutions were performed in water to prepare standard CS solutions (0, 100, 200, 500, 1000, and 2000 μg/mL), CSRM samples (1 mg/mL), and an ophthalmic solution (1 mg of CS/mL). One hundred microliters of CSRMs (1 mg/mL), an ophthalmic solution (1 mg of CS/mL), and standard CS solutions (100, 200, 500, 1000, and 2000 μg/mL) were mixed with 800 μL of Tris–acetate buffer (50 mM Trizma® base and 60 mM sodium acetate, pH 8.0), respectively. The samples were digested with 100 μL of chondroitinase ABC (1 mU/μL) at 37 °C for 1 h [29] or until the change of absorbance at 232 nm was no longer observed. After heating for 5 min and filtering on 0.45 μm filters (Millipore), the digestion mixtures (100 μL) were injected to the HPLC.

To confirm the purity of authentic CS, USP CS (Lot. F0B256) and NZP CS (1, 5, and 10 mg/mL) were digested by the procedure as described above and their total of unsaturated disaccharides were compared.

2.4. Quantitative and compositional analysis of CSRMs and CS in an ophthalmic solution

SAX-HPLC was performed to analyze the disaccharides derived from CSRMs and CS in an ophthalmic solution. Peaks of the disaccharides were confirmed with each authentic CS disaccharide (ΔDi-0S, ΔDi-6S, ΔDi-4S, and ΔDi-2,6diS) and the linear regression equation was calculated between the concentrations (μg/mL) of the authentic CS and the total peak areas of CS disaccharides. The disaccharides derived from CSRMs were quantified based on the calibration curves of the authentic sample and analyzed to determine the ratio of ΔDi-4S to ΔDi-6S to identify the source of CS.

2.5. Determination of the average molecular weight of CSRMs

The average MW of CSRMs was characterized by HPSEC. The MW standards used for the calibration curve were two types of CS having known MW (40,000 and 15,000 Da) and acharan sulfate-derived oligosaccharides ($n = 5$, MW = 2265 Da; $n = 10$, MW = 4530 Da; $n = 15$, MW = 6795 Da). Samples and MW standards (100 μg) were injected to HPLC and the calibration curve was established by plotting the logarithm of MW versus each retention time of MW standards [29].

2.6. Pretreatment and quantitation of CS from soft capsules and liquid preparations

Five soft capsules were individually weighed and transferred to 50 mL volumetric flasks. Authentic CS solution (25 mg/mL) was added to the soft capsules in 50 mL volumetric flasks in quantities of 0, 4, 8, 20, and 40 mL to obtain a spiked CS calibration curve. The total volume was adjusted into 50 mL by adding water and samples were dissolved after incubation at 37 °C and stirring. Sample solutions were accurately transferred to 100 mL separatory funnels and 50 mL of *n*-hexane was added to remove lipophilic ingredients. After mixing, the resulting two phases were left at room temperature for 6 h and the bottom aqueous layer was obtained. Two hundred microliters of the aqueous layer, 800 μL of water, and 1 mL of phenol–chloroform–isoamyl alcohol (25:24:1) were mixed in 2 mL tubes for 10 min and centrifuged at 15,000 × *g* for 10 min. One hundred microliters of the top aqueous layer were transferred to 1.5 mL tubes and dried by using the speed-vacuum dryer (N-Biotec Co, Korea) at 60 °C. In case of liquid preparations, 10 mL of test samples were mixed with authentic CS solutions (0, 2, 5, 10, 20, 30, and 60 mg/10 mL, respectively). One milliliter of each solution was extracted by 1 mL of phenol–chloroform–isoamyl alcohol (25:24:1), centrifuged, transferred, and dried under equal conditions just like pretreatment of soft capsules.

Dried samples were dissolved with 900 μL of Tris–acetate buffer, digested by chondroitinase ABC, and analyzed by SAX-HPLC as previously described (Section 2.3). CS content in soft capsules and liquid preparations was calculated from the spiked CS calibration curves and compared with the labeled amount (mg).

3. Results

3.1. Quantitative and compositional analysis of CSRMs and CS in an ophthalmic solution

CSRMs and CS in an ophthalmic solution were digested to reaction completion using excess chondroitinase ABC, which specifically degrades all linkages found within both chondroitin (and dermatan) sulfate without acting on other polysaccharides. The resulting CS disaccharides were quantitatively analyzed by SAX-HPLC. Except for CSRM B, all CSRMs consisted of ΔDi-0S, ΔDi-6S, and ΔDi-4S, and the quantity of high-sulfated disaccharides (ΔDi-2,6diS, ΔDi-4,6diS, ΔDi-2,4diS, ΔDi-2,4,6triS) was negligible. The CSRM B had di-sulfated disaccharides (14.27%) with ΔDi-0S, ΔDi-6S, and ΔDi-4S (data not shown) and their presence was confirmed based on their retention times compared to authentic CS disaccharides and by ^{1}H-NMR spectroscopy (data not shown). The CS content (%) in CSRMs and an ophthalmic solution versus the authentic CS was from 39.5 ± 0.1 to 105.6 ± 0.1 (Table 1) and 103.33 ± 1.17 (Table 3), respectively. CSRM B, C, E, F, G and L were very pure (> 90%),

Table 1
Quality evaluation of CSRMs on the basis of CS content, the ratio of ΔDi-4S to ΔDi-6S, source, and average MW

CSRM	CS (%) vs. Std NZP CS	ΔDi-4S/ ΔDi-6S ratio	Source	Average MW(Da)
A	42.7 ± 1.0	1.35	LA[a]	17,500
B	101.4 ± 1.4	0.26	SC	22,400
C	96.1 ± 0.4	1.45	LA	16,200
D	39.5 ± 0.1	3.10	LA	16,000
E	95.4 ± 0.2	4.22	LA	15,500
F	98.2 ± 0.5	3.30	LA	14,800
G	105.6 ± 0.1	1.60	LA	17,100
H	75.7 ± 1.7	1.32	LA	93,00
I	88.0 ± 0.5	2.21	LA	28,700
J	88.6 ± 1.1	2.16	LA	28,100
K	65.1 ± 0.2	3.16	LA	18,100
L	94.9 ± 1.3	3.44	LA	20,800
Std CS	100.0 ± 0.2	1.24	LA	17,900

[a] LA, land animals; SC, shark cartilage. Values are expressed as means ± SD ($n = 3$).

while CSRM A, D, H, I, J, and K were less than 90% purity. To confirm the sources of CS, the ratio of ΔDi-4S to ΔDi-6S was determined after enzymatic digestion of CSRMs. The results show that the CSRMs were originated from land animals probably (bovine or porcine) with the exception of CSRM B (Table 1). The source of CS in an ophthalmic solution was shown to be from shark cartilage, based on its disaccharide compositional pattern (a ratio of 0.22 for ΔDi-4S/ΔDi-6S). The peaks of other ingredients in an ophthalmic solution were detected at the washing step (0–4.155 min) after sample injection but no interference was observed at the retention times of CS disaccharides. Unlike the disaccharide pattern of NZP CS, a small quantity (7.63%) of ΔDi-2,6diS was present but other high-sulfated disaccharides (ΔDi-4,6diS, ΔDi-2,4diS, ΔDi-2,4,6triS) were not detected in CS from the ophthalmic solution (Fig. 2). The purity of NZP CS was 97.83 ± 0.10% based on that of USP CS (data not shown).

3.2. Determination of the average molecular weight of CSRMs

CSRMs were analyzed by HPSEC and their average MWs were calculated according to the calibration curve. All CSRMs had CS peak at 6.12–8.16 min and their average MWs ranged from 9300 to 28,700 Da (Fig. 3 and Table 1). Over half the CSRMs (A, C, D, E, F, G and K) had CS with MW range of 14,000–19,000 Da. While the average MW of four kinds (B, I, J, and L) was greater than 20,000 Da, CSRM H showed a MW less than 10,000 Da. The average MW of authentic CS was 17,900 Da. In case of CSRMs A and D, impurity peaks having the low MWs were also present (data not shown).

3.3. Pretreatment and quantitation of CS in soft capsules and liquid preparations

Through the pretreatment procedure, many ingredients mixed with CS were removed from samples. Oils in soft

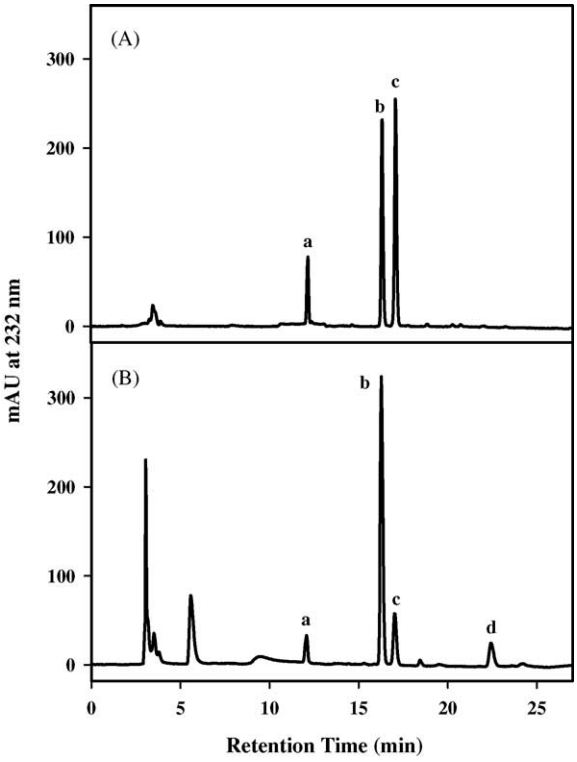

Fig. 2. SAX-HPLC analysis of CS disaccharides produced by enzymatic digestion (A) standard NZP CS; (B) ophthalmic solution; both samples (1 mg of CS/mL) were digested by chondroitinase ABC as described in the section 2.3. (a) ΔDi-0S (ΔUA-[l→3]-GalNAc); (b) ΔDi-6S (ΔUA-[1→3]-GalNAc-6S); (c) ΔDi-4S (ΔUA-[l→3]-GalNAc-4S); (d) ΔDi-2,6diS (ΔUA-2S-[1→3]-GalNAc-6S). The ophthalmic solution consists of CS, pyridoxine hydrochloride (HCl), dipotassium glycyrrhizinate, naphazoline HCl, dexpanthenol, propyl parahydroxybenzoate, cyanocobalamin, and methyl parahydroxybenzoate.

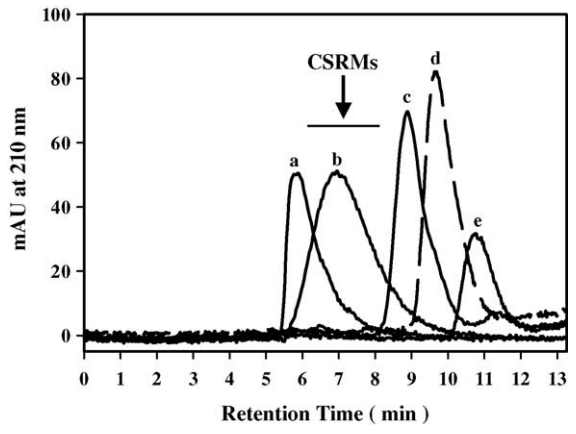

Fig. 3. MW standards and average MW range of CSRMs analyzed by HPSEC (a) CS (MW = 40,000 Da); (b) CS (MW = 15,000 Da); (c) acharan sulfate oligosaccharides ($n = 15$, MW = 6975 Da); (d) acharan sulfate oligosaccharides ($n = 10$, MW = 4530 Da); (e) acharan sulfate oligosaccharides ($n = 5$, MW = 2265 Da). The bar indicates the range of retention time of CSRMs.

Table 2
Recovery of spiked CS from formulated pharmaceuticals

Soft capsule			Liquid preparation		
Added (mg)	Determined (mg)	Recovery (%)	Added (mg)	Determined (mg)	Recovery(%)
300	310.62 ± 5.35	103.54 ± 1.78	10	10.32 ± 0.23	103.17 ± 2.34
500	483.94 ± 2.43	96.79 ± 0.49	20	19.71 ± 0.12	103.17 ± 2.34
1000	967.94 ± 5.29	96.79 ± 0.53	50	48.55 ± 0.91	97.10 ± 1.82

Values are expressed as means ± SD ($n = 3$).

Table 3
Quantitation of CS from pharmaceuticals

Sample	Labeled amount (mg)	Determined amount (mg)	Label claim (%)
Ophthalmic solution	2	2.07 ± 0.02	103.33 ± 1.17
Soft capsule	120	114.06 ± 0.03	95.05 ± 0.02
Liquid preparation	30	29.25 ± 0.13	97.48 ± 0.46

Values are expressed as means ± SD ($n = 3$). The labeled amount of CS in the ophthalmic solution, soft capsules, and liquid preparations was 2 mg/mL, 120 mg/capsule, and 3 mg/mL, respectively.

capsule solutions were selectively dissolved by *n*-hexane and capsule materials such as gelatin were denatured by phenol–chloroform, and precipitated, leaving them in the lower layer. Various herb extracts from liquid preparations were also separated from the top aqueous layer including CS by phenol–chloroform extraction. After drying the aqueous layer and digestion with chondroitinase ABC, the disaccharides derived from CS in each pharmaceutical formulation were quantified from the spiked CS calibration curves. The calibration curves for CS analysis in soft capsules and liquid preparations showed linearity ($r^2 \geq 0.999$) and the recovery (%) of spiked CS through all stages was 96.79 ± 0.53–103.54 ± 1.78 and 97.10 ± 1.82–103.17 ± 2.34, respectively (Table 2). The CS contents (mg) were 114.06 ± 0.03 per a soft capsule and 29.25 ± 0.13 per a liquid preparation (10 mL). The label claim (%) in soft capsules and liquid preparations was 95.05 ± 0.02 and 97.48 ± 0.46, respectively (Table 3). The peaks of other ingredients in pharmaceuticals were also detected at the washing step (0–4.155 min) but no interference was observed at the retention times of CS disaccharides (Fig. 4).

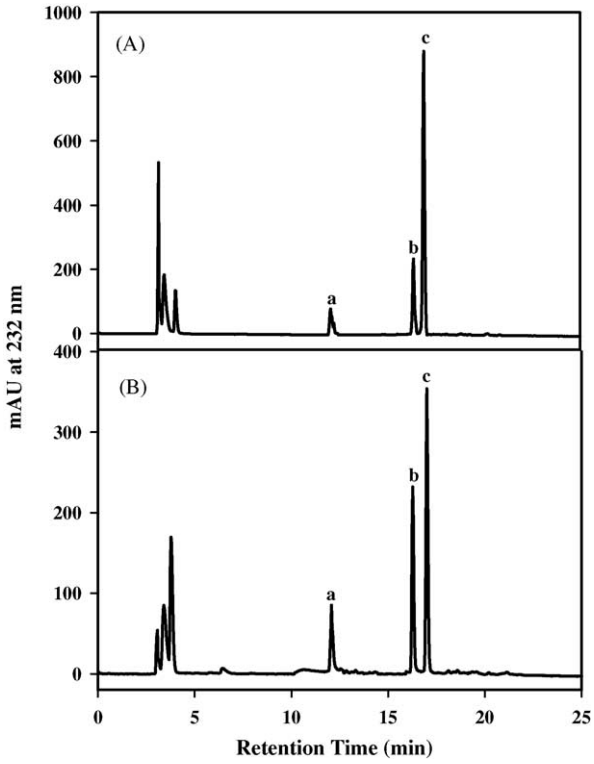

Fig. 4. SAX-HPLC analysis of the disaccharides derived from CS in soft-capsules and liquid preparations after pretreatment (A) soft-capsules; (B) liquid preparations; both samples were digested by chondroitinase ABC after pretreatment as described in the section 2.6. (a) ΔDi-0S (ΔUA-[1→3]-GalNAc); (b) ΔDi-6S (ΔUA-[1→3]-GalNAc-6S); (c) ΔDi-4S (ΔUA-[1→3]-GalNAc-4S). The soft capsule contains CS, tocopherol acetate, fursultiamine, nicotinamide, pyridoxine HCl, pantothenic acid, riboflavin butyrate, y-oryzanol, cyanocobalamin, methyl parahydroxybenzoate, propyl parahydroxybenzoate, garlic extract. The liquid preparations contain CS, pyridoxine HCl, cyanocobalamin, nicotinamide, sodium pantothenate, thiamine HCl, carnitine HCl, anhydrous caffeine, riboflavin sodium phosphate, diluted ethanol extract of Ginseng Radix and Oriental Bezoar, Royal Jelly, Epimedium Herba extract, sodium benzoate, and propyl parahydroxybenzoate.

4. Discussion

In many countries, CS-pharmaceuticals are produced as various forms such as tablets, hard capsules, soft capsules, ophthalmic solutions, and liquid preparations. There has been a significant increase in the number of pharmaceuticals and nutraceuticals containing CS. However, the quality of CSRMs, the main ingredient of these products, remains in question and a previous report showed that the serious lack in the label claim was found in some nutraceuticals containing CS and/or glucosamine sold in USA [30]. Hence, the accurate and practical analytical methods for quality control of CS have been required to evaluate the quality, the contents, and the properties.

There are several analytical methods for the quantitative determination of CS. Many spectrophotometric methods including carbazole assay have been mainly used for quality control of CS. Recently, a photometric titration method [25]

and a HPSEC method [26] were reported. Although these analytical methods are easy and convenient, they also have certain disadvantages. In the case of spectrophotometric methods, the presence of impurities having molecular similarity to constituents of CS is serious problem. During the purification of CS, impurities such as carbohydrates, proteins, and reagents may be not completely removed from the CSRMs. Furthermore, it is possible for some suppliers to add other materials into CSRMs to satisfy the claimed purity taking advantage of the weakness of spectrophotometric methods. Many molecules having negative charge groups can affect the UV absorbance through their combination with color forming reagents. The photometric titration assay uses cetylpyridinium chloride (CPC), which binds with CS to be formed CS-CPC complex and this complex is precipitated out and detected by the automatic calorimeter [25]. However, this method is not also free from interference by the impurities having sulfate or other negative charges and the automatic calorimeter for CPC titration is not widely available to many pharmaceutical companies. HPSEC methods cannot distinguish other materials from CS if the MW of impurities was similar with that of CS.

Manufacturers of CSRMs obtained their products from animal tissues. After several purification steps, the quality of final CSRMs is evaluated depending on their quality control, mainly by spectrophotometric methods. Hence, it is possible that although low-quality CSRMs have reduced amounts of CS, impurities affecting UV absorbance can result in these methods overestimating CS content. When we tested 12 different CSRMs, the CS content in several of these CSRMs was not consistent with the label claim in their specifications. As an additional experiment, NMR analysis was undertaken to confirm the integrity of CS structure and the presence of impurities, showing that low-quality CSRMs contained impurities such as maltose oligosaccharides, mannitol, and lactose (data not shown). The analysis of the disaccharides derived from CS after enzymatic digestion is highly specific, more accurate than other assay methods and provides not only the actual content of CS but also the additional information such as composition and origin of the CSRM. Moreover, this method is not affected by other carbohydrates clearly differentiating it from spectrophotometric methods (data not shown). Another merit of the present method is that the component ratio of disaccharides offers the information on the CS source. Recently, consumers and pharmaceutical companies have had increased interest in the source of pharmaceutical and/or nutraceutical ingredients. Furthermore, the safety of these ingredients, purified from land animal tissues, has been in question because epidemics, including mad-cow disease, foot-and-mouth disease, and hog cholera, have become a serious issue. Hence, identifying the source of the raw materials used is important and disaccharide analysis after enzymatic digestion can provide this information.

The quantitative analyses of CS from pharmaceuticals have been mainly focused on tablets and hard capsules. To enhance the nutritional efficacy, however, the addition of various ingredients including multi-vitamins with CS has become very popular. However, many ingredients mixed with CS can interfere in quantitation of CS from pharmaceuticals. The peaks associated with the ingredients in an ophthalmic solution did not interfere with those of CS disaccharides (Fig. 2). In the CS analysis of soft capsules, however, many problems were discovered in the absence of sample solution pretreatment. These solutions have high viscosity so they were not filtered through membrane filters and can solidify at low temperature. Many herb extracts in liquid preparations can be detected together with CS. Moreover, various ingredients, soft capsule materials, and herb extracts can shorten the lifetime of HPLC columns and prevent the accurate analysis. Our pretreatment method makes it possible to remove many ingredients from pharmaceutical sample solutions.

CS is widely used as a nutraceutical and pharmaceutical raw materials. As the number of products containing CS increases, stricter and more accurate evaluation should be required for the manufacture of high quality products. Disaccharide analysis using SAX-HPLC should be useful for evaluation of the quality of CSRMs as a pharmaceutical and nutraceutical ingredient. The pretreatment method followed by enzymatic digestion makes it possible to quantify CS content in soft capsules and liquid preparations and should be applicable for the quality control of CS. The present methods can be applied to confirm the purity and label claim of CS in raw materials, pharmaceuticals, and nutraceuticals.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2004.12.015.

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