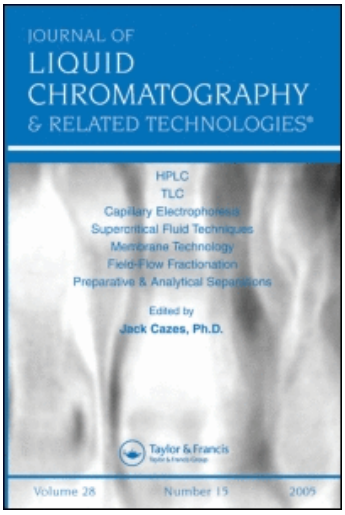

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DETERMINATION OF CHONDROITIN SULFATE IN NUTRITIONAL SUPPLEMENTS BY LIQUID CHROMATOGRAPHY

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DETERMINATION OF CHONDROITIN SULFATE IN NUTRITIONAL SUPPLEMENTS BY LIQUID CHROMATOGRAPHY

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ABSTRACT

A size exclusion chromatography (SEC) method for the determination of chondroitin sulfate (CS) in nutritional supplements was developed and validated based on USP guidelines. Samples were dissolved in water and analyzed using a Phenomenex BioSep-SEC-S2000 column with an isocratic mobile phase consisting of 0.1 M sodium phosphate at pH = 7.0. CS was detected with a photodiode array detector at 207 nm. Data were collected from 200-360 nm.

The method proved highly reliable with respect to standard performance characteristics. The method aids in convenient quality control in the growing CS market.

INTRODUCTION

Many health supplements have appeared on the market recently that are intended to help combat osteoarthritis by building up levels of chondroitin sulfate in articular cartilage.[1,2] Administration of external sources of purified chondroitin sulfate provide a high level of bioavailability in the body.[3] Glycosaminoglycans, including chondroitin sulfate, are large biopolymers that consist of repeating disaccharide units made up of aminosugars.

In addition to treating the symptoms of osteoarthritis,[4,5] chondroitin sulfate has been shown to modify the progression of the disease.[6,7,8] None of the other GAG biopolymers including hyaluronic acid, dermatan sulfate, and heparan sulfate have shown efficacy in combating osteoarthritis when given orally.

Recently, supplements containing chondroitin sulfate, particularly those higher in the 4-sulfate isomer, have been shown to inhibit degradation enzymes that are responsible for cartilage deterioration, and to possibly provide material for the body to synthesize new GAG biopolymers.[7,9] Chondroitin sulfate is available from many sources including bovine, ovine, porcine, and shark cartilage. To date, clinical trials have primarily used the bovine form, which is predominantly the 4-sulfate isomer, rather than shark cartilage, which is predominantly the 6-sulfate isomer.

Clinical and experimental trials have documented efficacy and safety of a specific combination of glucosamine HCl with low molecular weight chondroitin sulfate for the protection of cartilage and relief of pain associated with arthritis in humans and animals.[10-17] The same combination has also shown to be disease modifying by preventing the progression of osteoarthritis.[7]

Nutritional supplement therapy is gaining popularity for the treatment of arthritis. However, since these products are classified as dietary supplements, the label claim, molecular weight, and, thus, presumably efficacy, varies greatly.[18,19] While many methods for analyzing chondroitin sulfate have been described, convenient methods are lacking for analyzing chondroitin sulfate in product matrices.

Several analytical methods for separating and quantitating chondroitin sulfate and other GAG's have focused on digesting the biopolymer with various lyase enzymes[20-25] or solvolysis[26] that cleave the polysaccharide into the disaccharide units. Usually, these methods are designed to detect the components at low levels in blood, plasma, and urine samples. The disaccharides have then been separated using reverse-phase HPLC,[20] anion-exchange resins,[27,28] hydroxyapatite columns,[29] and capillary electrophoresis.[30]

A variety of detection methods has also been employed, such as, UV spectroscopic,[23] fluorimetric,[22,31] suppressed conductivity,[28] chemiluminescence,[32] and pulsed amperometric[27] and colorimetric titrations.[33-37]

This article describes a fast and reliable procedure in which chondroitin sulfate is separated from and quantified in nutritional supplements. The method is useful for monitoring product content in a quality control environment. The chondroitin sulfate is dissolved in water directly from the product supplement matrix and separated by a size exclusion chromatography (SEC) column in conjunction with UV/Vis detection.

The method appears suitable for health supplement matrices such as tablets, capsules, oil base, and powder form.

EXPERIMENTAL

Apparatus

Chromatography analysis was performed using a Varian Model 9012 Solvent Delivery System equipped with a Varian Model AI-200 Autosampler and a ThermoSeparations Products SM5000 Photodiode Array Detector using a Phenomenex BioSep-SEC-S2000 column, 300 x 7.8mm.

Samples and standards were introduced using a 50µL injection size, and chondroitin sulfate (CS) was eluted using a sodium phosphate buffer at 0.1M, pH adjusted to 7.0 with 50% NaOH solution, and monitored at 207 nm using LCTalk Chromatography Software. A summary of these conditions is shown in Table 1.

Robustness testing was performed using a second system. A Varian Model 9010 Solvent Delivery System with a Varian Model 9090 Autosampler and a Varian Model 9050 UV/Vis detector were used while other conditions were held constant.

Reagents and Chemicals

Chondroitin Sulfate, 95% reference grade was obtained from Bioiberica, Barcelona Spain through Nutramax Laboratories® Inc., Edgewood MD. Nutritional supplement samples, Cosamin® DS and Cosequin® Equine powder concentrate, were also obtained through Nutramax Laboratories®. HPLC grade water was prepared from a Millipore Milli-Q water purification system.

Table 1

Conditions

Mobile Phase	0.1 M sodium phosphate, pH = 7.0
Flow Rate	0.8 mL/min
Injections	50 µL partial-loop
Column	Phenomenex BioSep-SEC-S2000 with security guard cartridge replaced daily
Detector	UV @ 207, PDA data collected 200 - 360 nm

Sodium phosphate monobasic, reagent grade, was obtained from Fisher Chemical Co. while Sodium Hydroxide solution, 50% in water was purchased from Aldrich Chemical Co.

Preparation of Chondroitin Sulfate Standards for Analysis

About 0.2 g chondroitin sulfate reference material was weighed into 50.0 mL volumetric flask. The flask was then filled halfway with water and sonicated for 10 minutes and mixed until all material dissolved. The volumetric flask was then diluted to volume with water at room temperature. This stock solution was used to prepare solutions of approximately 0.20, 0.40, 0.60, and 0.80 mg/mL chondroitin sulfate in water.

Preparation of Nutritional Supplement Samples for Analysis

An amount of sample theoretically equivalent to approximately 40 mg chondroitin sulfate was weighed from a homogeneous preparation of multiple (5 to 10) dosage units. The material was added into a 100 mL volumetric flask and filled halfway with water. The sample was sonicated for 20 minutes and mixed. Often, some of the nutritional supplement matrix did not dissolve in water. The volumetric flask was then diluted to volume with water at room temperature. Samples were then filtered using 0.45 micron PTFE filters.

RESULTS

The method was validated using the raw material source from Bioiberica (95% chondroitin sulfate) and a finished product which contains this raw material source Cosamin® DS, (Nutramax Laboratories® Inc). This CS source was used in the registered European drug CS (Condrosulf® IBSA, Lugano, Switzerland). It is also the material that has been shown clinicaly safe and effective in European and US trials.

In addition to the above mentioned CS, Cosamin® DS contains glucosamine hydrochloride, manganese ascorbate, and magnesium stearate. The validation data appears in Table 2 and shows that the performance of the method to meet all reasonable criteria.

To determine method accuracy, linearity, and range, ten (10) samples of known chondroitin sulfate were prepared in the range of 0.024 g to 0.092 g (60% to 230% of the target). Over the entire range tested, CS recoveries ranged from 95.2% to 100.7% with an average recovery of 98.7%. A residual plot of this data is shown as Figure 1. The balanced distribution of the data around zero indicates no recovery bias throughout the demonstrated working range. The correlation coefficient (R^2) of these measurements was greater than 0.999.

Table 2

Validation Data

Analytical Parameter	Measurement
Accuracy	98.7% Recovery
Precision	2.48% RSD of 10 Samples
Linearity	> 0.999 Correlation (R^2)
Range	60 to 230% of Target
Specificity	Interferences < 1% of Target
Robustness	104.8% of First Analysis

Method precision was determined by analyzing ten (10) replicate samples of Cosamin® DS according to the method. Calculations based on peak height rather than peak area allowed for higher precision in the measurements. This is apparently due to the lack of baseline resolution between the CS and the very low molecular weight materials in the matrix.

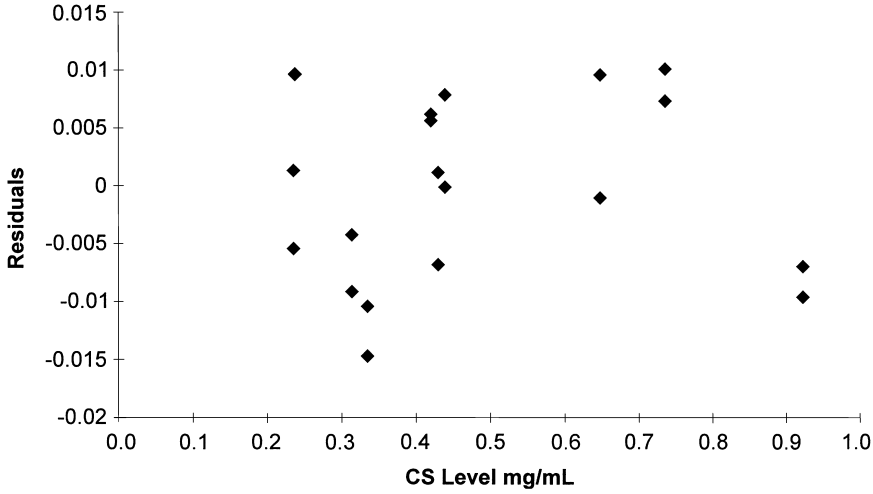

Figure 1. A residual plot of CS recoveries. Plotted is the deviation of the CS recovery from average recovery as a function of CS level.

A typical chromatogram of Cosamin® DS is shown in Figure 2. Results ranged from 353.2 mg/g to 379.6 mg/g with an average of 364.2 mg/g and a 2.48% RSD.

In determining method robustness, three (3) replicate samples of Cosamin® DS were prepared and analyzed according to the method. Results ranged from 369 mg/g to 392 mg/g, with an average of 382 mg/g and a 2.78% RSD which is equivalent to 100% of label claim.

Magnesium stearate, manganese ascorbate, and glucosamine hydrochloride were analyzed by the method to determine if these components interfered with the CS assay. No interfering peaks were noted above a level of 3 mg/g at the retention time of CS. This corresponds to less than 1% of the target level of CS.

The Cosamin® DS product is a capsule. The method was also tested on Cosequin®, powder concentrate matrix that included microcrystalline cellulose. The microcrystalline cellulose did not apparently affect the assay since results were above the industry accepted 95% of label claim.

DISCUSSION

Most methods in the literature for analyzing CS and other glycoaminoglycans (GAGs) have used pre-column preparations, including enzymatic digestion, acid digestion, and solvolysis as well, as pre- and post-column derivitization for fluorescence and chemiluminescence detection. These methods can be difficult and time consuming, making them impractical for a quality control laboratory.

Furthermore, these methods are susceptible to inaccuracies due to variations in the CS,[12] such as sulfated position, 4-sulfate or 6-sulfate, as well as, any oversulfated portion of the gluronic acid or galactosamine portion. However, the method described here uses sample preparation without digestion or derivitization.

The SEC method works on the principle of separating materials by molecular weight. This method is suitable for most chondroitin sulfate nutritional supplement products, because chondroitin sulfate is often the only high molecular weight material present in those formulations. Moreover, the initial water extraction allows for the separation of the water-soluble chondroitin sulfate from water-insoluble polymeric materials such as common fillers.

It is suspected, although not shown in this report, that other cartilage components such as the GAGs; hyaluronic acid, dermatin sulfate, or keratin sulfate

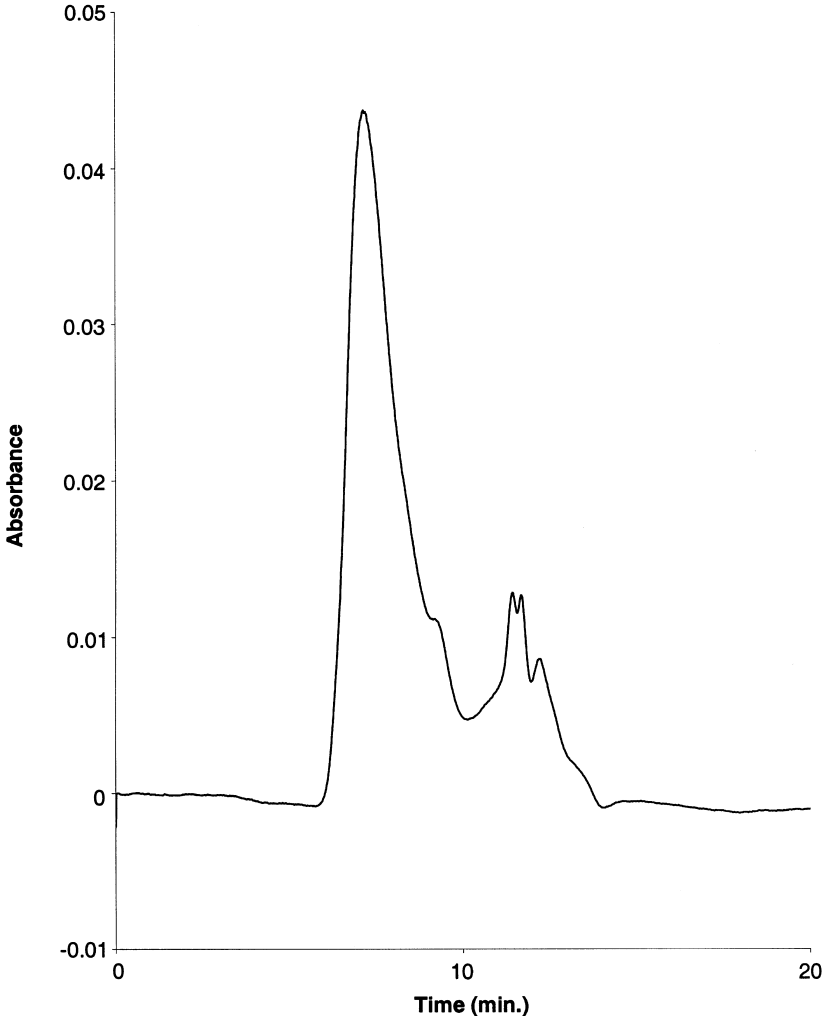

Figure 2. Typical chromatogram of Cosamin® DS Nutramax Laboratories® Inc.

would cause an interference or overestimation of the level of chondroitin sulfate present in a nutritional supplement. Chondroitin sulfate is extracted from cartilage containing animal tissues. If not well purified, it is likely that it can be contaminated with the aforementioned components.

With the possibility of additional government regulation of the nutritional supplement, industry, validated quality control methods for assaying nutritional supplement activities will be required.

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