Application Note

Analysis of Chondroitin Sulfate Sodium Salt by SEC

Introduction

There are several types (A to E) of chondroitin sulfate (MW 20,000 ~ 50,000) (one of which is a mucopolysaccharide) which are found in animal connective tissue - cartilage and skin. Chondroitin sulfate is widely used in the manufacture of health foods and medicines. In this application note Chondroitin sulfate (sodium salt) was analyzed using a SEC (Size Exclusion Chromatography) column with an exclusion limit of 300,000 molecular weight (Pullulan) and detection using refractive index (RI detector). A Pullulan calibration curve was created from a standard mixture using ChromNAV with the optional GPC Calculation Program and the resulting molecular weights calculated from the Pullulan standard are described below.

JASCO Refractive Index Detector at
www.jascoinc.com

Keywords

Chondroitin sulfate, SEC, RI detector, GPC Calculation Program

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Experimental

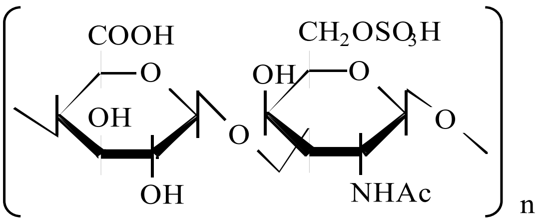
Equipment

Pump:	PU-2080
Degasser:	DG-2080-53
Column Oven:	CO-2060
Autosampler:	AS-2057
Detector:	RI-2031

Conditions

Column:	Shodex Asahipak GF-510 HQ (7.5 mmI.D. x 300 mmL, 5) µm
Eluent:	0.04 M Sodium dihydrogen phosphate anhydrous-0.06 M Disodium hydrogen phosphate anhydrous
Flowrate:	0.6 mL/min
Column Temp.:	30 °C
Injection Volume:	50 µL
Calibration Standards for SEC:	Shodex STANDARD P-82 (Pullulan) 0.05 % each eluent

Structure



Chondroitin Sulfate C

PL-STD1		PL-STD2	
Grade	Mp	Grade	Mp
P-100	107,000	P-200	200,000
P-20	21,100	P-50	47,100
P-5	5,900	P-10	9,600

Sample: 0.1% Chondroitin sulfate C sodium salt in eluent

Results

Fig. 1 shows the chromatograms of Pullulan (PL), standard mixture for molecular weight calibration and chondroitin sulfate sodium salt.

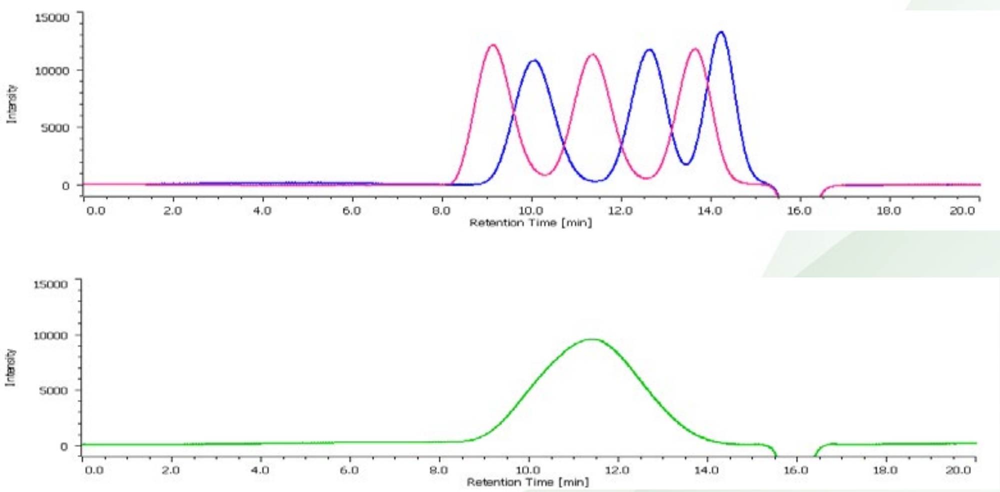


Fig. 1. Chromatograms of Pullulan (PL), Standard Mixture for Molecular Weight Calibration and Chondroitin Sulfate Sodium Salt
(Each value on the chromatograms is Mp (Peak top molecular weight).)

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Fig. 2 shows molecular weight calibration curve created with Pullulan as a standard mixture.

Fig. 3 shows a chromatogram of chondroitin sulfate sodium salt and molecular weight calibration curve.

Fig. 4 and Table 1 shows molecular weight distribution calculation curve and the result of molecular weight calculated with Pullulan respectively.

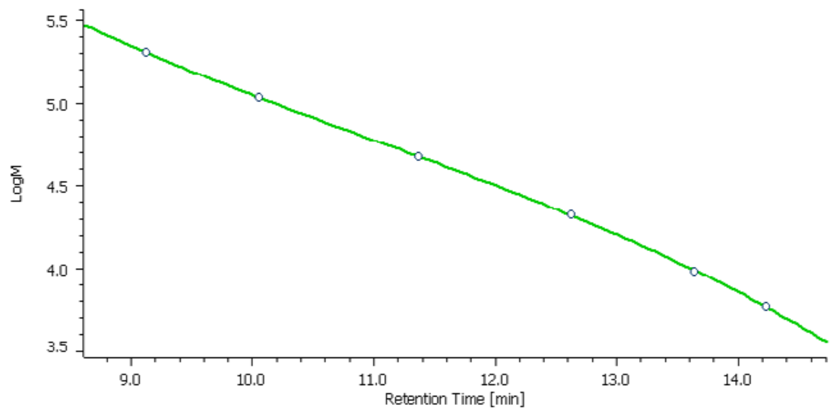

Fig. 2. Molecular Weight Calibration Curve Created with PL Standard Mixture

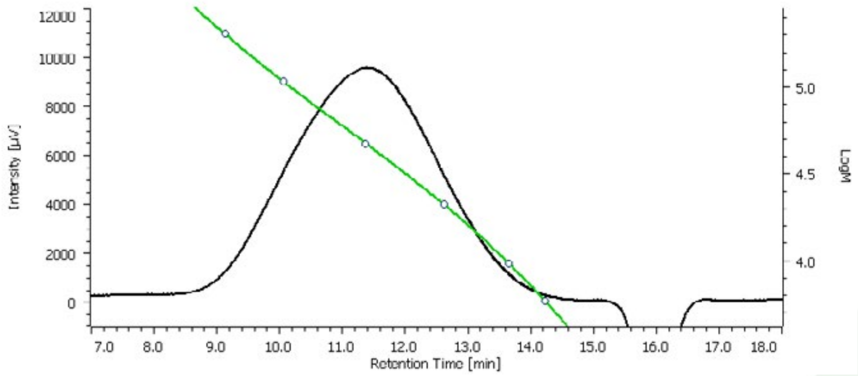

Fig. 3. Chromatogram of Chondroitin Sulfate Sodium Salt and Molecular Weight Calibration Curve
(The figure in blue represents Mp calculated with Pullulan.)

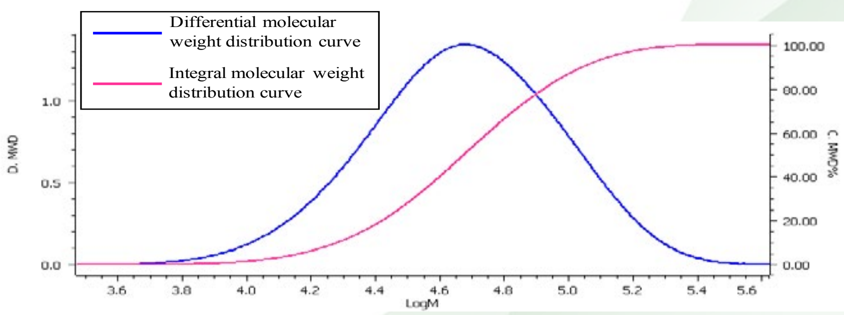

Fig. 4. Molecular Weight Distribution Curve of Chondroitin Sulfate Sodium Salt

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Table 1. Pullulan Converted Molecular Weight of Chondroitin Sulfate Sodium Salt

Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw
46812	36668	57914	85947	57914	1.58	1.48

Hint

<Molecular weight calibration curve>

Shows the relationship between retention volume (elution time) and molecular weight, which is created to estimate the molecular weight of the sample from its retention volume (elution time).

<Molecular weight distribution curve>

The integral molecular weight distribution curve shows the relationship between molecular weight (logarithmic value) and the percentage of the total of a specific molecular weight.

The differential molecular weight distribution curve is used to plot the molecular weight (logarithmic value) on the x-axis against the weight fraction differentiated from a logarithmic value of molecular weight (dw/d(logM)) on the y-axis. When normalized, the use of this curve makes it possible to compare a chromatogram and a molecular weight distribution derived using different columns and measurement conditions.

<Molecular weight averages and polydispersity>

The molecular weight averages of polymer materials obtained by size exclusion chromatography (SEC) includes the number-average (Mn), weight average (Mw), z-average (Mz), and viscosity-average (Mv) molecular weights. These averages are defined by the following expressions. The distribution of these molecular weight averages bears in general a relationship such as Mn ≤ Mv ≤ Mw ≤ Mz. In case of Mn = Mv = Mw = Mz, there is no molecular weight distribution (mono-dispersed).

Mn (Number-average molecular weight):

$$Mn = \frac{\sum_{i=1}^{\infty}(N_i \times M_i)}{\sum_{i=1}^{\infty} N_i}$$

Mv (Viscosity-average molecular weight):

$$Mv = \left[\frac{\sum_{i=1}^{\infty}\left(N_i \times M_i^{a+1}\right)}{\sum_{i=1}^{\infty}(N_i \times M_i)}\right]^{1/a}$$

Mw (Weight average molecular weight):

$$Mw = \frac{\sum_{i=1}^{\infty}\left(N_i \times M_i^2\right)}{\sum_{i=1}^{\infty}(N_i \times M_i)}$$

Mz (Z-average molecular weight):

$$Mz = \frac{\sum_{i=1}^{\infty}\left(N_i \times M_i^3\right)}{\sum_{i=1}^{\infty}\left(N_i \times M_i^2\right)}$$

* N_i represents the number of molecules for *i* component of molecular weight M_i and *a* represents an index of Mark-Houwink-Sakurada equation.

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