Analysis of Chondroitin Sulfate Sodium Salt by SEC

Introduction

There are five kinds of chondroitin sulfate (MW 20,000 ~ 50,000) which is one of mucopolysaccharide, such as A~E, contained in connective tissues of animal's cartilagines and skin, and they are used for drug medicines and healthy food. By using a column with exclusion limit of 300,000 molecular weight (Pullulan) for SEC (Size Exclusion Chromatography), chondroitin sulfate sodium salt was analyzed with an RI detector. The calibration curve was created with Pullulan as standard mixture by ChromNAV GPC Calculation Program (Optional) and the result calculated based on Pullulan converted molecular weight is shown below.

Keyword : Chondroitin sulfate, SEC, RI detector, GPC Calculation Program

Experimental

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

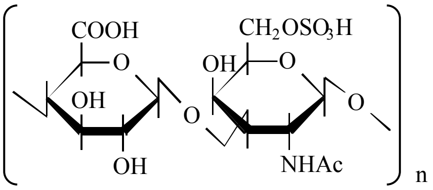
Conditions

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
Flow rate: 0.6 mL/min
Column temp.: 30 ºC
Injection volume: 50 µL
Calibration standards for SEC: Shodex STANDARD P-82 (Pullulan)
0.05 % each eluent

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

Structure



Chondroitin Sulfate C

Results

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

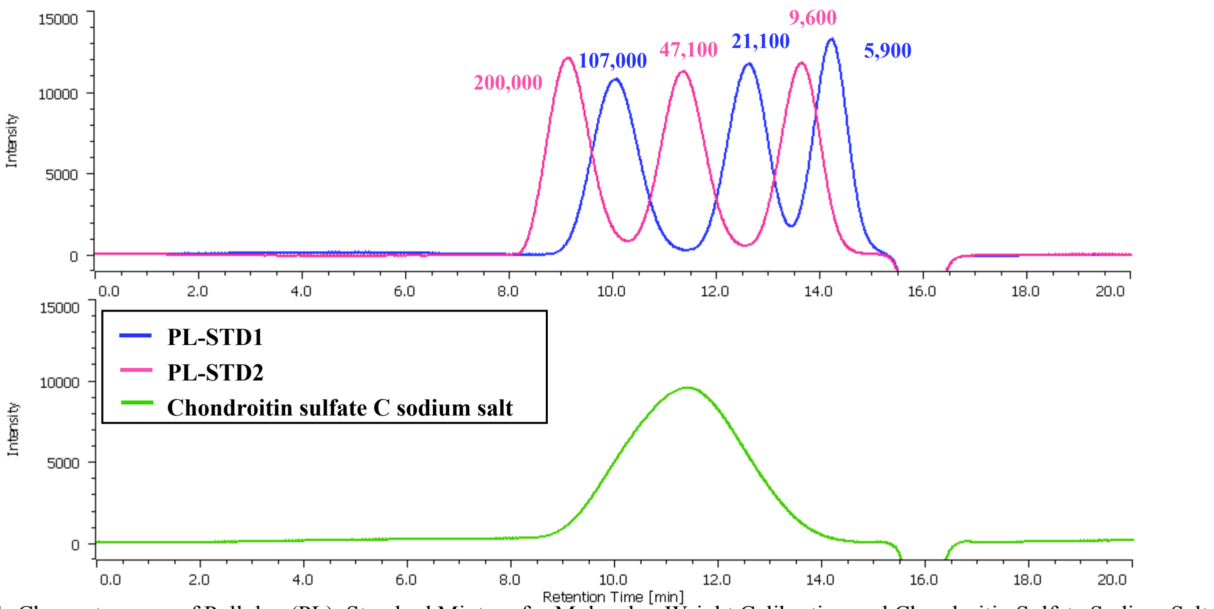


Fig. 1. Chromatograms of Pullulan (PL), Standard Mixture for Molecular Weight Calibration and Chondroitin Sulfate Sodium Salt (Each value on the chromatograms is Mp (Peak top molecular weight).)

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.

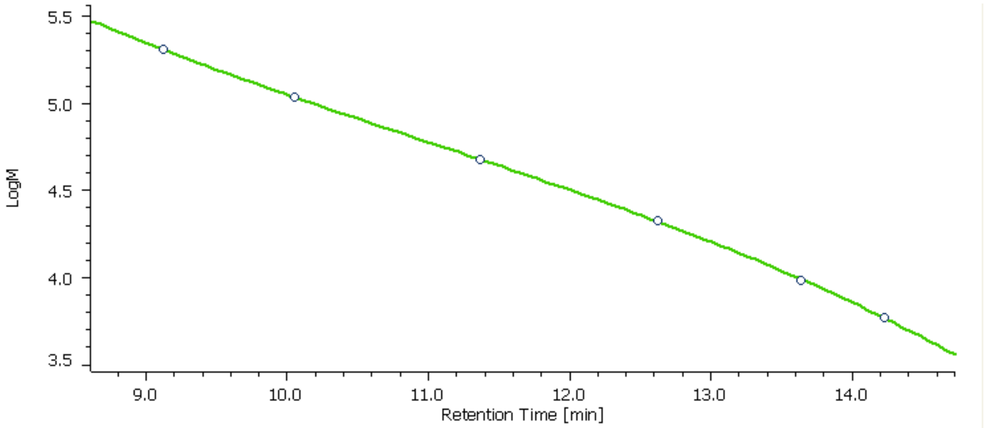


Fig. 2. Molecular Weight Calibration Curve Created with PL Standard Mixture

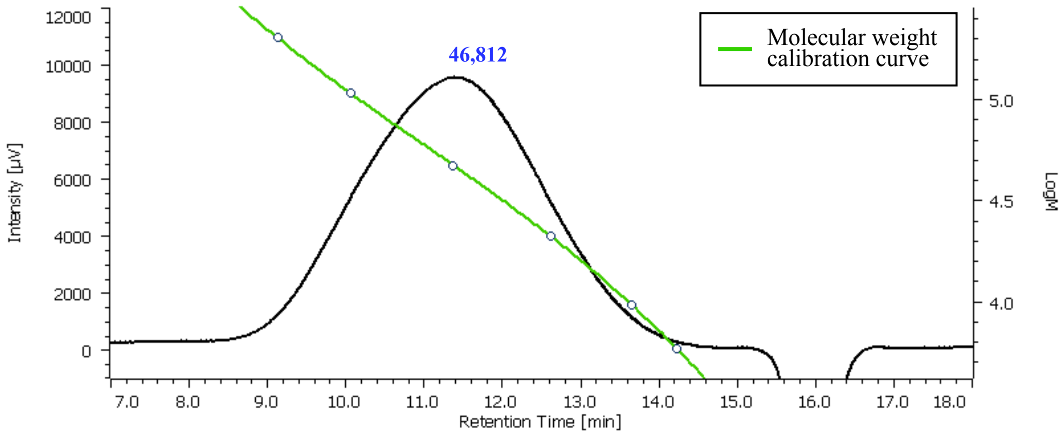


Fig. 3. Chromatogram of Chondroitin Sulfate Sodium Salt and Molecular Weight Calibration Curve
(The figure in blue represents Mp calculated with Pullulan.)

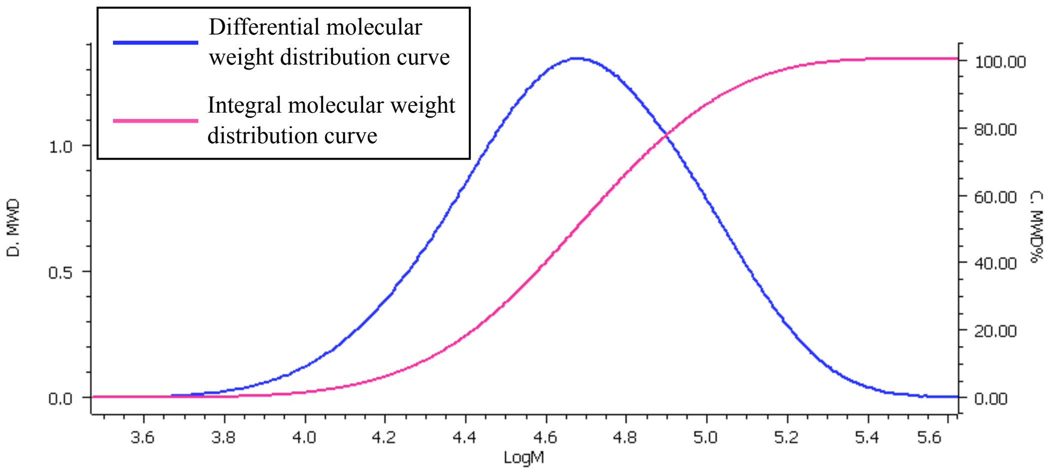


Fig. 4. Molecular Weight Distribution Curve of Chondroitin Sulfate Sodium Salt

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>

This is to show the relationship between retention volume (elution time) and molecular weight, which is created to estimate the molecular weight of the sample from the retention volume (elution time) of the sample eluted from the column.

<Molecular weight distribution curve>

Integral molecular weight distribution curve is to show the relationship between molecular weight (logarithmic value) and a percentage against the total to indicate how much percentage of some molecular weight occupies against the total.

Differential molecular weight distribution curve is a curve where the molecular weight (logarithmic value) is plotted on the abscissa axis and the value of weight fraction differentiated by logarithmic value of molecular weight (dw/d(logM)), on the ordinate axis. When normalized, such curve makes it possible to compare a chromatogram and a molecular weight distribution under the 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 $Mn \leq Mv \leq Mw \leq Mz$. In case of $Mn = Mv = Mw = Mz$, there is no molecular weight distribution (monodispersed).

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.

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512