SHIMADZU APPLICATION NEWS

LAAN-A-LC-E024

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L322

Analysis of Hyaluronic Acid

Hyaluronic acid is an acidic mucopolysaccharide in which D-N-acetylglucosamine and D-gluconic acid are alternately bonded, and is known to be distributed throughout the body in tissues such as the skin, tendons, muscles, cartilage, blood vessels and brain; with a molecular weight in excess of 1,000,000.
Since hyaluronic acid exists naturally in living organisms, its high biocompatibility is of interest, and it has been used in applications such as surgical procedures. Since hyaluronic acid gradually degrades in vivo, there is a growing need for hyaluronic acids with higher molecular weights, which remain effective for longer periods. Hyaluronic acid also has a high moisturizing effect, and thus is used as a cosmetic additive.
Here, we would like to introduce examples of the use of size-exclusion chromatography in the analysis of hyaluronic acid.

■ Analysis of Standard Samples

Fig.1 shows the structural formula of hyaluronic acid. When using size-exclusion chromatography for the analysis of macromolecules that contain ionic dissociation groups, these ionic groups within the molecule will repel each other, causing the molecule to expand. These ionic interactions can be suppressed by adding a salt in the mobile phase. In particular, in the case of a hydrophilic molecule such as polysaccharides, the existence of any sort of ionic groups will frequently cause pattern deformation and unstable measurement results. In this example, sodium sulfate was used as the salt. The analytical conditions are shown in Table 1.
Fig.2 shows an example of the analysis of sodium hyaluronate derived from rooster comb. The sample is dissolved to give a 0.1% concentration in the 50mM aqueous solution of sodium sulfate used as the mobile phase.

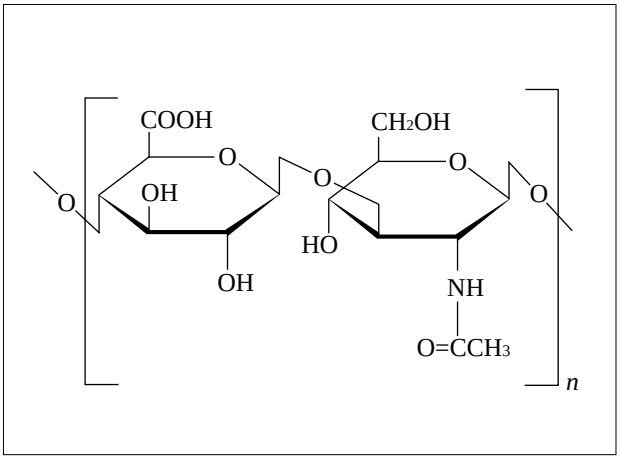

Fig.1 Structural Formula of Hyaluronic Acid

Table 1 Analytical Conditions

Column	: Shodex OHpak SB-806M HQ × 2
Mobile Phase	: 50mM Na_2SO_4 Aqueous Solution
Flow Rate	: 1.0mL/min
Column Temp.	: 40˚C
Detection	: RID-10A
Inj. Volume	: 50µL

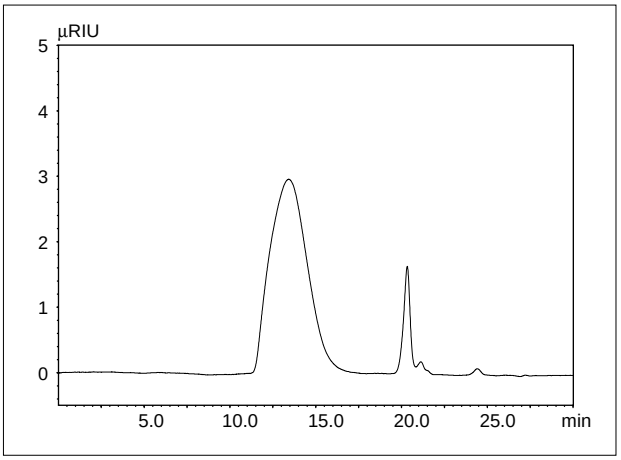

Fig.2 Chromatogram of Sodium Hyaluronate from Rooster Comb

Fig.3 shows an example of the analysis of sodium hyaluronate derived from human umbilical cord. In the same manner as with Fig.2, the sample is dissolved to give a 0.1% concentration in 50mM aqueous solution of sodium sulfate.

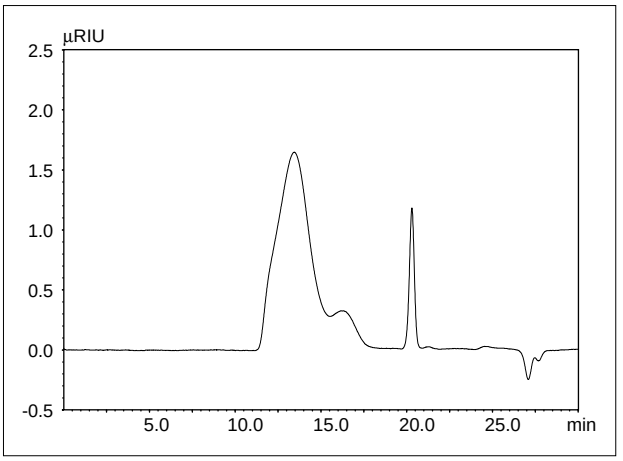

Fig.3 Chromatogram of Sodium Hyaluronate Derived from Human Umbilical Cord

■ Analysis of Soft Drinks and Cosmetics

We analyzed some cosmetics and soft drinks that claim to contain hyaluronic acid. The analytical conditions are shown in Table 1. Chromatograms are shown for a soft drink in Fig.4, cosmetic A in Fig.5, and cosmetic B in Fig.6. Cosmetic A was analyzed after being diluted 10-fold in a 50mM aqueous sodium sulfate solution used as the mobile phase. The soft drink and cosmetic B were analyzed without dilution, but after filtration through a 0.45µm pore filter. The peaks between 11 and 17minutes are presumed to be hyaluronic acid.

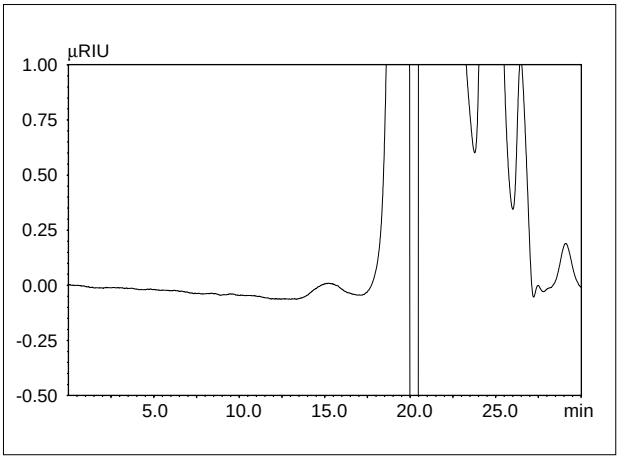

Fig.4 Chromatogram of a Soft Drink

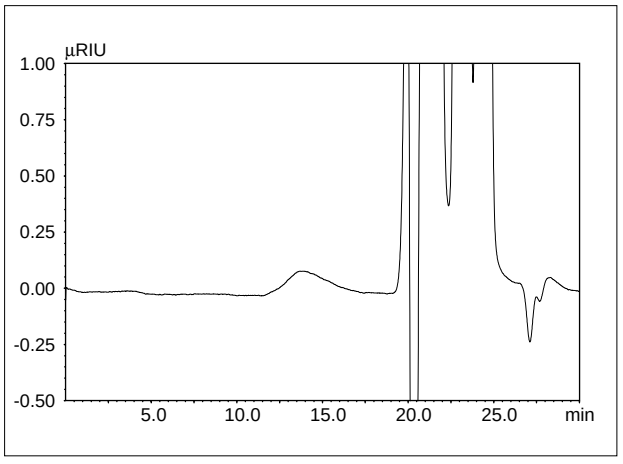

Fig.5 Chromatogram of Cosmetic A

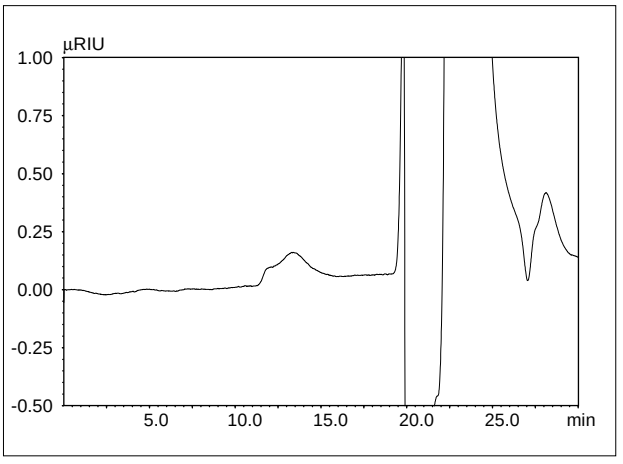

Fig.6 Chromatogram of Cosmetic B

* Data presented here was not acquired using instruments approved under the Japanese Pharmaceutical Affairs Law.

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