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Application News

No. L567

High Performance Liquid Chromatography

Analysis of Sodium Chondroitin Sulfate by ELSD-LT III

Chondroitin sulfate, which is contained in a lot of tissues such as cells and cartilages is a kind of mucopolysaccharides. Chondroitin sulfate is often included in eye drops and supplements as sodium chondroitin sulfate (hereinafter called as SCS) due to its positive effect to corneal protection and improvement of joint pain. SCS cannot be detected by UV-VIS spectrophotometric (UV) detector, which is often used in HPLC because SCS has very limited UV absorption. Therefore, refractive index detector (hereinafter called as RID) or evaporative light scattering detector (hereinafter called as ELSD) is used to detect SCS. ELSD is a highly versatile detector because it can be used to detect almost all compounds except volatile ones. It provides reduced analysis time and improved sensitivity compared to those of analyses in which RID is used due to applicability to gradient elution. Therefore, ELSD is used in analyses of various substances such as sugars and lipids.

Here, SCS in eye drops was analyzed by Nexera™ series with ELSD-LT III as a detector.

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Analysis of SCS in Standard Solution by ELSD

Table 1 shows the analytical conditions of SCS (Manufactured by Tokyo Chemical Industry Co., Ltd. , CAS No. 9082-07-9) by ELSD. Fig. 1 shows the chromatogram of SCS standard solution. Here, SCS was analyzed using a non-porous ODS column, which is suitable for protein analysis with gradient elution followed by ELSD. Wide function, a new feature of ELSD-LT III, automatically optimizes a parameter that are related to sensitivity and a single method file can be used for data acquisition regardless of sample concentration, from low to high.

Table 1 Analytical Conditions (ELSD)

System	: Nexera lite
Mixer	: Mixer MR 20 µL *1
Column	: Imtakt Presto FF-C18 (150 mm x 4.6 mm I.D., 2 µm)
Mobile Phase	: A) Water/Triethylamine/Acetic Acid=100:1.1:0.5 B) Acetonitrile
Time Program	: B conc. 5% (0 min) →55% (7 min) →100% (7.10 – 9 min) →5% (9.10 min - 18 min)
Flow Rate	: 0.35 mL/min
Column Temp.	: 35 °C
Injection Vol.	: 1 µL
Vial	: LabTotal for LC 1.5 mL, Glass*2
Detection	: ELSD-LT III
Gain	: Wide
Filter	: 4 sec
Drift Tube Temp.	: 100 °C
Nebulizer Gas	: N_2
Gas Pressure	: 350 kPa

*1 P/N: 228-72652-41 、 *2 P/N: 227-34001-01

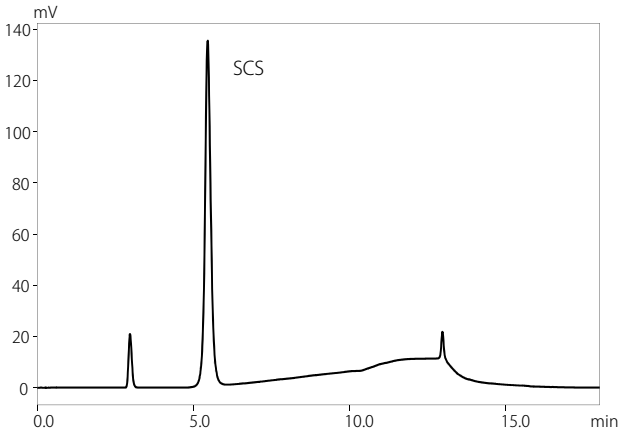

Fig. 1 Chromatogram of SCS Standard Solution (1000 mg/L) (ELSD)

Linearity and Repeatability

Calibration curve of SCS was created using four different concentrations of 250, 500, 750, 1000 mg/L. The response of ELSD was plotted on double logarithmic axes because the logarithm of ELSD response is in proportion to the logarithm of concentration. Fig. 2 shows the calibration curve. Table 2 shows the repeatability. The repeatability was confirmed using repeated analyses at 250 mg/L. The calibration curve showed excellent linearity ,with r^2 of more than 0.9999. The repeatability of retention time and area also showed good results. The relative standard deviations (%RSD, n=6) were 0.11 about retention time and 2.08 about area.

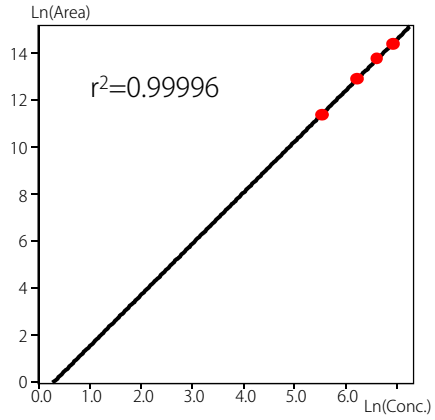

Fig. 2 Calibration Curve (ELSD)

Table 2 Repeatability (250 mg/L Standard Solution) (ELSD)

Compound	Retention Time (%RSD)	Area (%RSD)
Sodium Chondroitin Sulfate	0.11	2.08

Analysis of SCS in Eye Drops by ELSD

This Analysis was carried out under the same analytical conditions shown in Table 1. Eye drops were diluted 10 times with water and filtered with a 0.2 µm membrane filter to analyze. Fig. 3 shows the chromatogram of the eye drops. The determination result of SCS in the ten time diluted eye drops was 629.4 mg/L. This value was calculated using the calibration curve in Fig. 2. It is strongly recommended that the ingredient SCS of the sample was used for creating the calibration curve due to the difference of molecular weight distribution over every production lot of SCS.

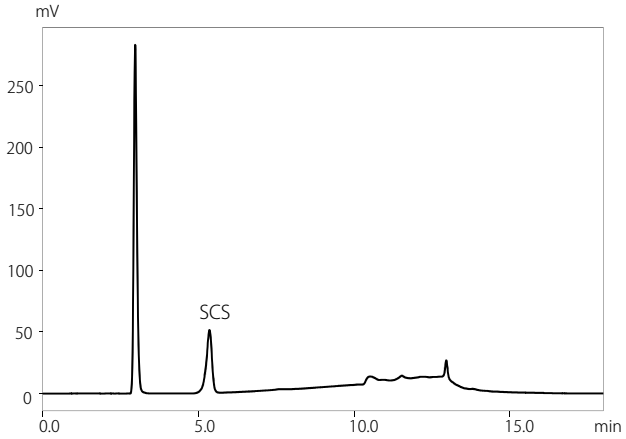

Fig. 3 Chromatogram of Eye Drops(Diluted 10 times) (ELSD)

Analysis of SCS in Eye Drops by RID

Table 3 shows the analytical conditions of SCS in eye drops by RID. A water-based SEC (GFC) column was used to analyze SCS. 50 µL was injected in order to certainly detect the SCS peak. The pretreatment of eye drops was the same as described above. Fig. 4 shows the chromatogram. Components of the eye drops were not able to be separated because gradient elution was impossible to be applied to RID is used due to its character. On the other hand, the analysis by ELSD has advantages, for example excellent separation, shorter analysis time, and good sensitivity so on, because gradient elution can be applied .

Table 3 Analytical conditions (RID)

System	: Nexera lite
Mixer	: Mixer MR 180 µLII*1
Column	: Shodex OHpak SB-806M HQ (300 mm x 8.0 mm I.D.,13 µm)
Mobile Phase	: 50 mmol/L Sodium Sulfate aq. : Methanol=95 : 5
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 50 µL
Vial	: LabTotal for LC 1.5 mL, Glass*2
Detection	: RID-20A
	Polarity : +
	Cell Temp. : 40 °C
	Response : 1.5 sec

*1 P/N: 228-72652-44、*2 P/N: 227-34001-01

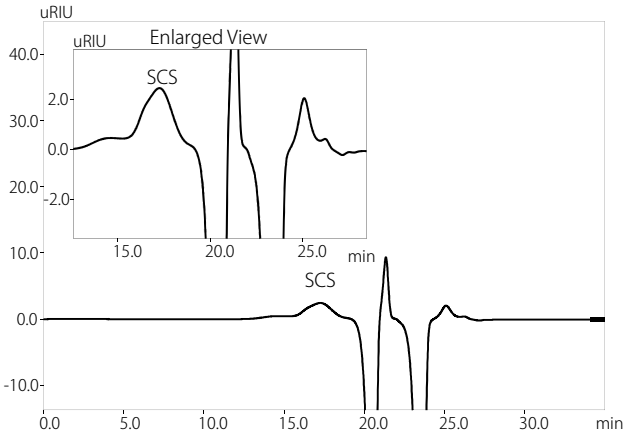

Fig. 4 Chromatogram of eye drops (diluted 10 times) (RID)

Conclusion

Analysis of SCS in eye drops were carried out. In this article, a non-porous ODS column was used for separation and ELSD-LT III was used as a detector. In the analysis in which ELSD was used, use of gradient elution afforded to the separation the components of the eye drops and the determination of SCS.
Wide function, a new feature of ELSD-LT III, automatically optimized a parameter that were related to sensitivity and a single method file were able to be used for data acquisition regardless of sample concentration, from low to high.

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