

Stability and recovery issues concerning chondroitin-sulfate disaccharide analysis

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We aimed to discover the potential decomposition products formed in solution during storage. We used two different chromatographic methods: the standard gradient (see 4.2 chapter in the main body) and an altered gradient for spreading the early eluting decomposition products. MS conditions were the same. Only those species were considered decomposition products that had different retention times from the parent species using both methods. (Same retention time means identical chromatographic behavior, thus most probably the structure is the same and the ion is formed by decomposition in the mass spectrometer.)

The altered chromatographic conditions were the following. A 250 μm x 10.5 cm self-packed GlycanPac AXH-1 capillary column was used, the column temperature was set to 45°C, and the flow rate was adjusted to 8 $\mu\text{L}/\text{min}$. Eluent A was 10 mM ammonium formate in 75:25 v/v ACN:water (pH 4.4); Eluent B was 65 mM ammonium formate in 75:25 v/v ACN:water (pH 4.4). Starting from 0% B, the eluent ratio changed in 4 min to 2% B, and then in 2 minutes to 60% B. As a washing step, the composition was held elevated to 100% B and held for 4 minutes and it was followed by a 5-minute-long equilibration at the initial composition.

We discovered that the CS disaccharides can suffer a major sulfuric acid elimination, in accordance with former NMR studies (1). A peak for N-acetyl galactosamine was also observed which is derived from the decomposition into monosaccharide units (Fig. S1).

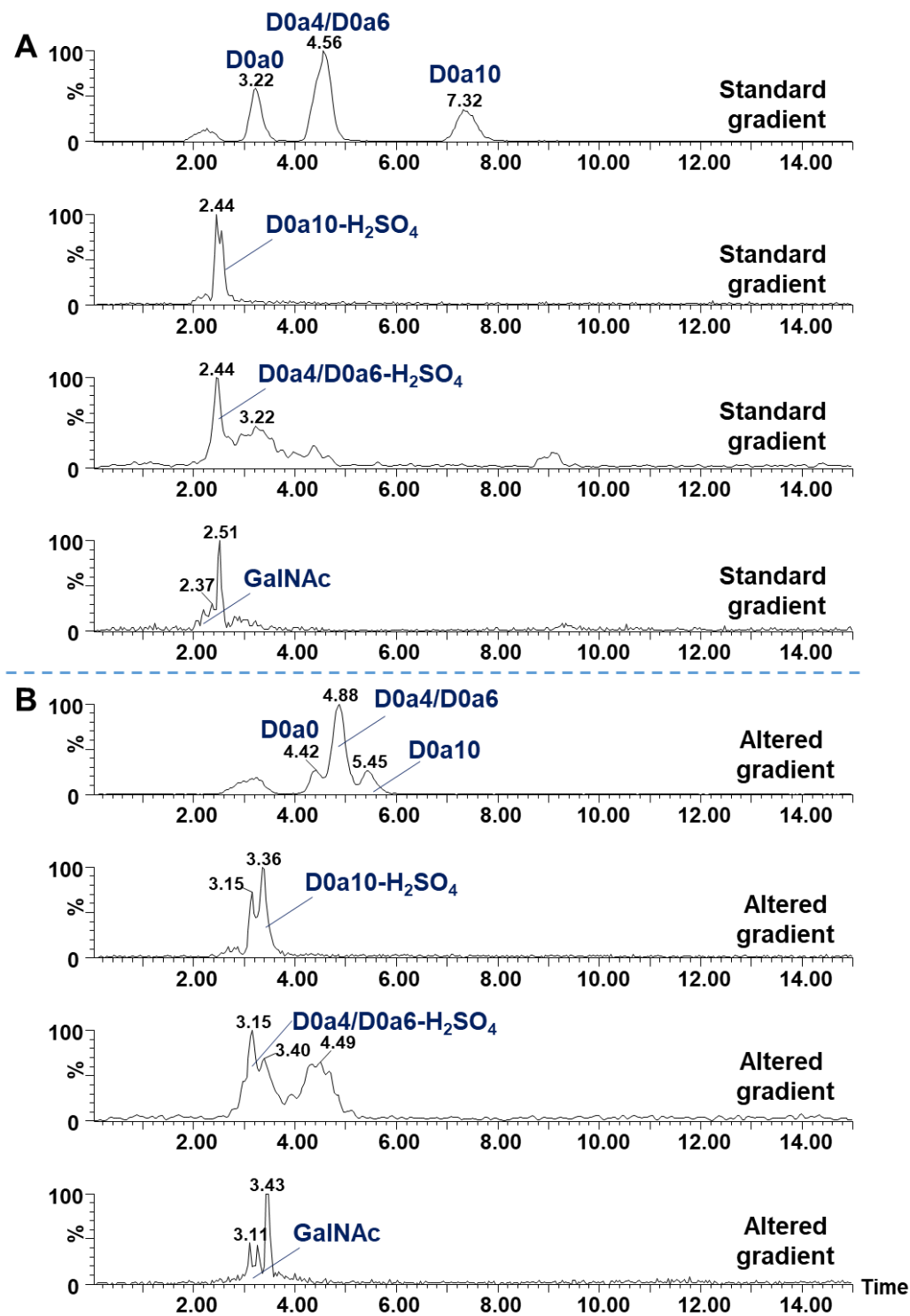


Fig. S1 Extracted ion chromatograms (EICs) of CS disaccharides and their most abundant decomposition products. A: EICs using the standard gradient, B: EICs using an altered gradient for spreading the early eluting components

1. Volpi N, Mucci A, Schenetti L. Stability studies of chondroitin sulfate. Carbohydrate research. 1999;315(3-4):345-9.