FIGURE LEGENDS TO SUPPLEMENTAL FIGURES

FIGURE S1. Purity evaluation of LMWHA and HMWHA by FACE. LMWHA and HMWHA preparations were analyzed before and after treatment with chondroitinases ABC and ACII in combination. Δdi- and oligosaccharides (6-16 mers) of HA were used as standards (*lanes 1 and 2*, respectively). Intact (before treatment with chondroitinases) LMWHA and HMWHA are shown in *lanes 3 and 4*, respectively. No HA-oligosaccharides were detected in both preparations. Digestion products of LMWHA and HMWHA, identified as Δ-disaccharide, are shown in *lanes 5 and 6*, respectively. No CS-derived Δdisaccharides were detected, indicating the absence of CS from both HA preparations. Lane 7 shows the HA- and CS-derived Δ-disaccharides obtained following chondroitinases digestion of the ophthalmic solution Viscoat®, which contains both HA and CS.

FIGURE S2. Evaluation of the LMWHA and HMWHA homogeneity. Intact LMWHA and HMWHA preparations were analyzed using a reversed polarity CE methodology. As shown, LMWHA preparation migrated as single peak indicative of a homogeneously charged population. The same profile was obtained for the HMWHA preparation. No CS and HA oligosaccharides were detected in both LMWHA and HMWHA.

FIGURE S3. Identification of HA oligosaccharides obtained following digestion of LMWHA and HMWHA with hyaluronidase. LMWHA and HMWHA preparations were analyzed by FACE before and after treatment with *Streptomyces hyaluronidase*. Δdi- and oligo-saccharides (6-16 mers) of HA were used as standards (*lanes 1 and 2*, respectively). Intact LMWHA and HMWHA are shown in *lanes 3 and 4*, respectively. HA preparations at the concentrations of 5 and 10 μg/ml for LMWHA (*lanes 5 and 6*) and HMWHA (*lanes 7 and 8*) were digested with hyaluronidase. Mixed species (4-mers + 6-mer of HA) were identified for both HA digests.

FIGURE S4. Identification of LMWHA oligosaccharide species following digestion with hyaluronidase by capillary electrophoresis. The digest of LMWHA obtained following treatment with hyaluronidase was analyzed by reversed polarity capillary electrophoresis on an uncoated fused extended light path silica capillary at 25^{o}C, using a 50 mM phosphate buffer (pH=3.0) at 30 kV, and detection of migrated HA oligosaccharides at 200nm. The electropherogram of digested LMWHA shows the presence of two homogenous charged populations of 6-mers (4) and 4-mers (5) migrating at 14.8 and 16.6 min, respectively. The identification of these populations was achieved comparing their migration times with those of standard HA oligosaccharides as well as following analyses of the digest spiked with the various sized oligosaccharide standards. Arrows indicate the migration positions of the various HA oligomers: 12-mers (1), 10-mers (2), 8-mers (3), 6-mers (4), 4-mers (5) and Δ-disaccharide (6).

Figure S1

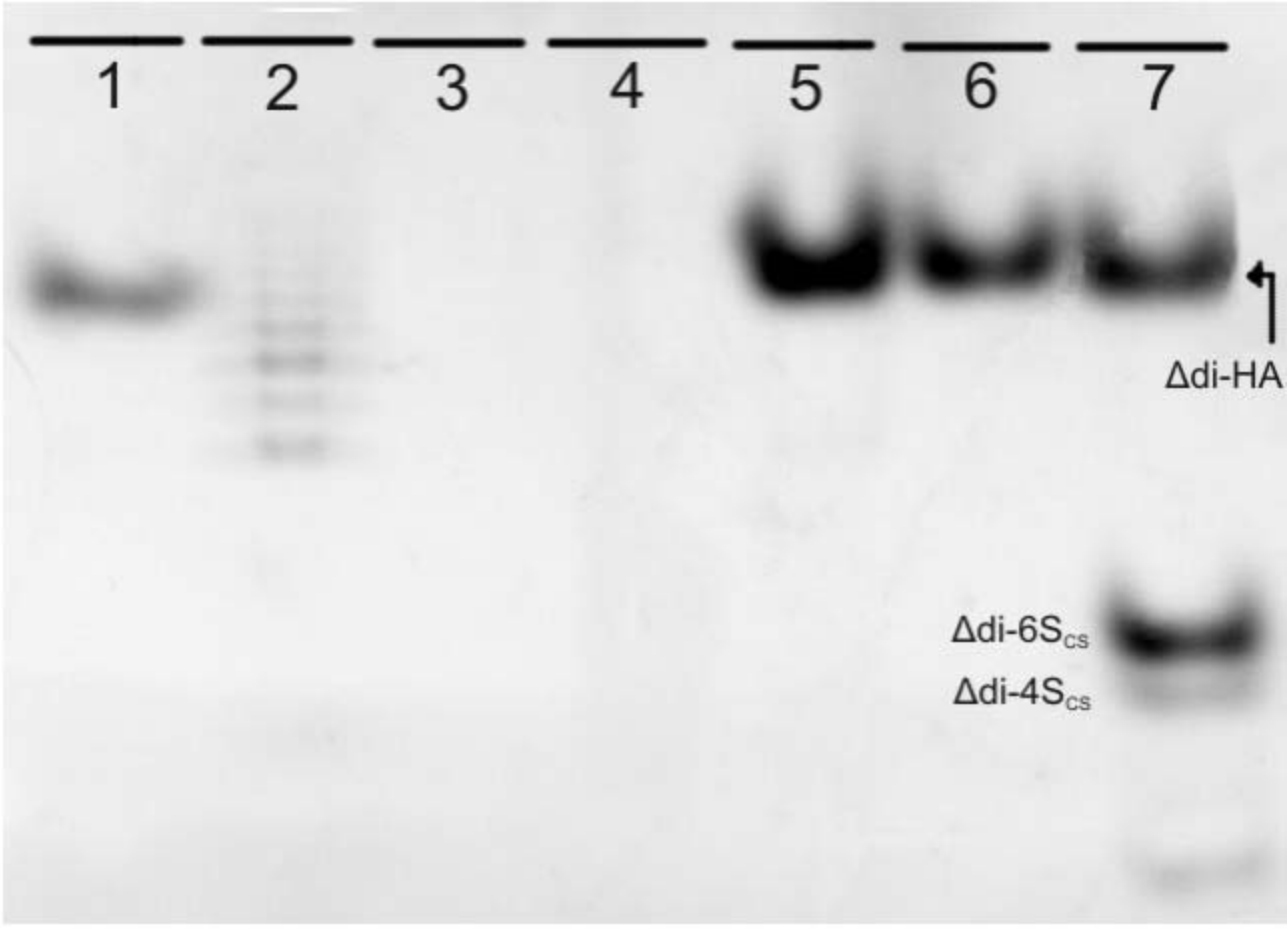


Figure S2

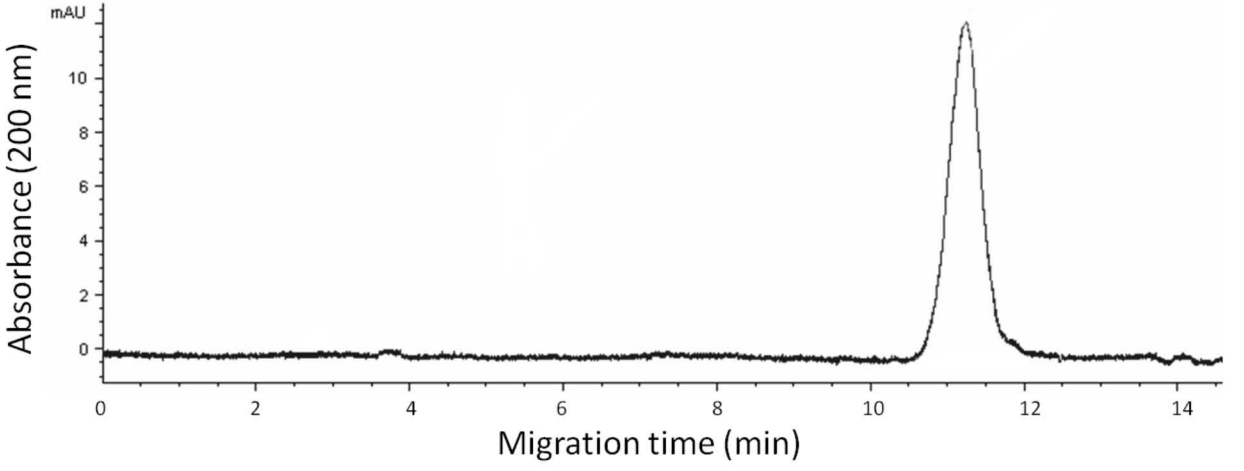

mAU
Absorbance (200 nm)
10
8
6
4
2
0
0
2
4
6
8
10
12
14
Migration time (min)

Figure S3

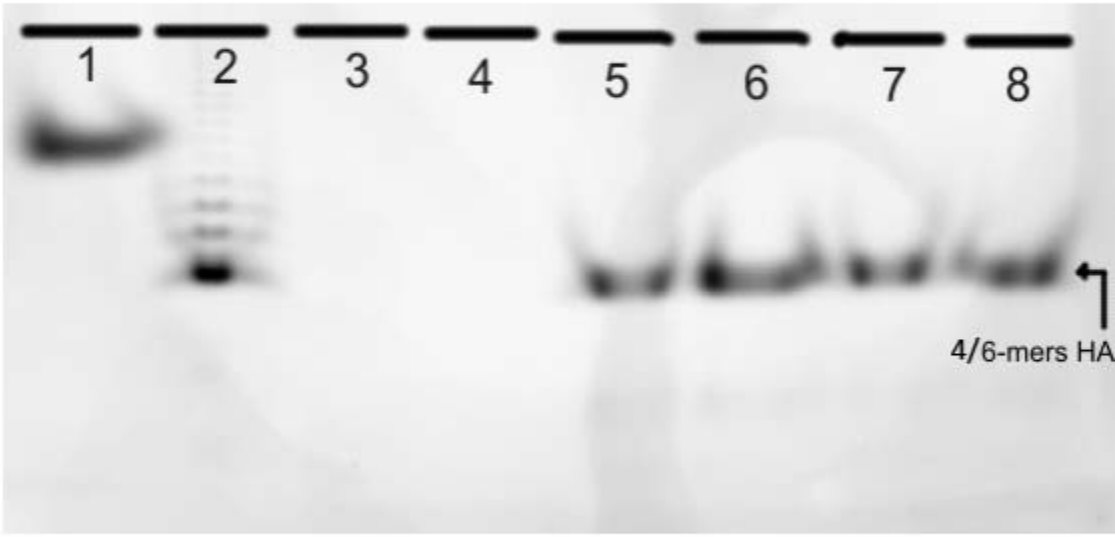
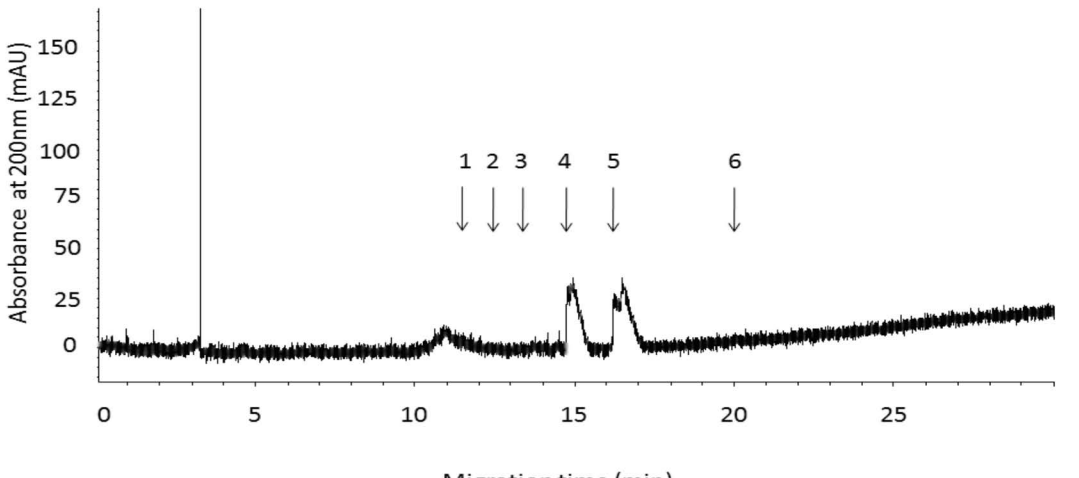


Figure S4


Absorbance at 200nm (mAU)
150
125
100
75
50
25
0
1 2 3 4 5 6
0
5
10
15
20
25
Migration time (min)