Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary information

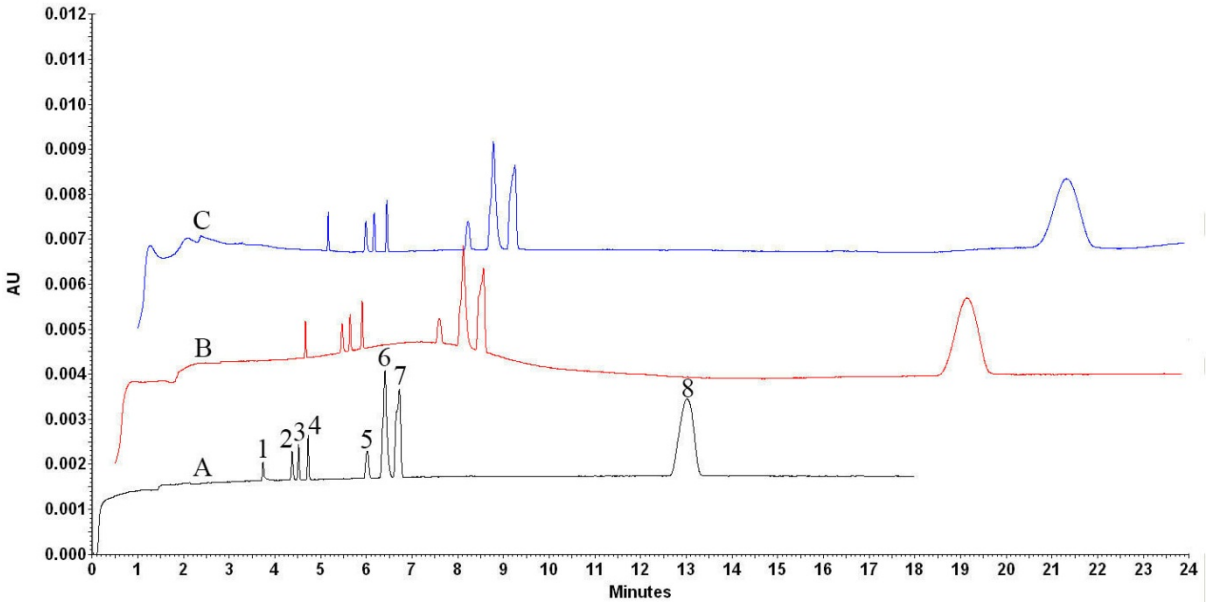


**Fig. 1.** Electropherogram of a standard mixture of chondro-disaccharides. Concentrations: ΔDi-triS, ΔDi-di$S_D$, ΔDi-di$S_B$, ΔDi-di$S_E$, ΔDi-UA2S: 25 μg/mL; ΔDi-6S, ΔDi-4S, ΔDi-0S: 125 μg/mL. Fused silica capillary (total length: 60.2 cm, effective length: 50 cm, i.d. 50 μm). Capillary temperature: 37 ℃. Sample temperature: 4 ℃. Injection: pressure injection at 0.5 psi×5s. Detection wavelength: 232 nm. Voltage: -30 kV. Peaks identification: (1) ΔDi-triS, (2) ΔDi-di$S_D$, (3) ΔDi-di$S_B$, (4) ΔDi-di$S_E$, (5) ΔDi-UA2S, (6) ΔDi-6S, (7) ΔDi-4S, (8) ΔDi-0S. (A) BGE: pH 3.0, 170 mM Tris phosphate buffer. (B) BGE: pH 3.0, 180 mM Tris phosphate buffer. (C) BGE: pH 3.0, 190 mM Tris phosphate buffer.

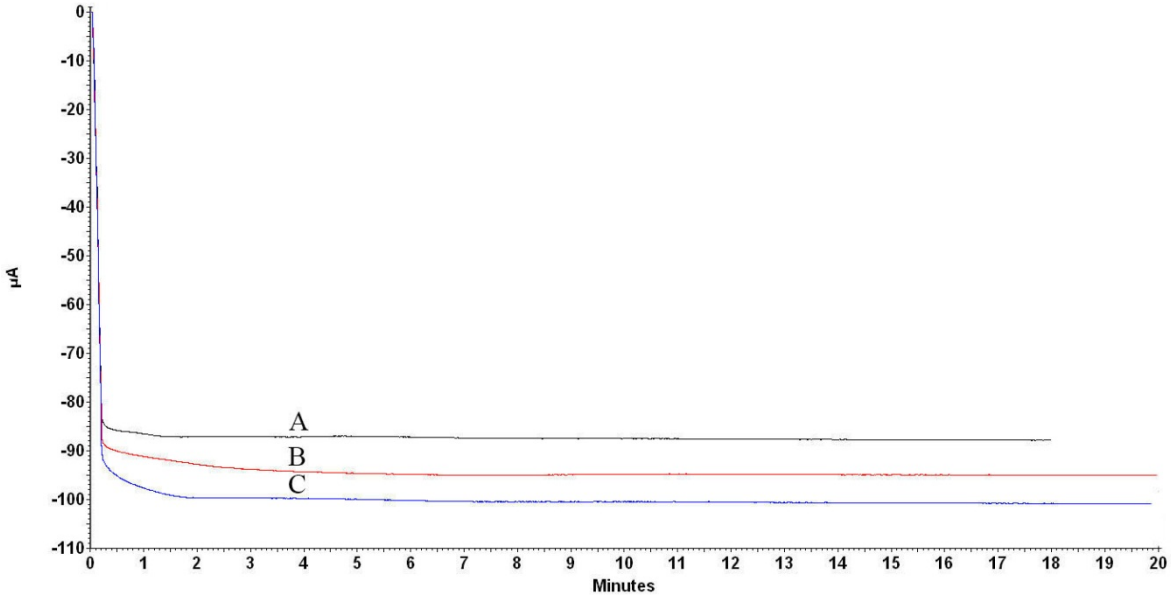


**Fig. 2.** Current of different concentrations of pH 3.0 Tris-phosphate buffer. (A) BGE: pH 3.0, 170 mM Tris phosphate buffer. (B) BGE: pH 3.0, 180 mM Tris phosphate buffer. (C) BGE: pH 3.0, 190 mM Tris phosphate buffer. Other conditions were as in Fig. 1.

Table 1. Precision of the migration time of eight standard disaccharides obtained by CZE separation

| Disaccharides | △UA→ GalNAc Na | △UA→ GalNAc-6SNa$_2$ | △UA→ GalNAc-4SNa$_2$ | △UA-2S→ GalNAcNa$_2$ | △UA → GalNAc-4S,6SNa$_3$ | △UA-2S→ GalNAc-6SNa$_3$ | △UA-2S→ GalNAc-4SNa$_3$ | △UA-2S→ GalNAc-4S-6SNa$_4$ |
|---|---|---|---|---|---|---|---|---|
| run | | | | time(min) | | | | |
| 1 | 3.625 | 4.983 | 5.063 | 5.358 | 7.633 | 8.299 | 8.346 | 14.654 |
| 2 | 3.653 | 4.892 | 5.022 | 5.376 | 7.625 | 8.388 | 8.387 | 14.589 |
| 3 | 3.598 | 4.876 | 5.123 | 5.283 | 7.592 | 8.243 | 8.291 | 14.572 |
| 4 | 3.623 | 4.986 | 5.072 | 5.391 | 7.592 | 8.289 | 8.241 | 14.983 |
| 5 | 3.665 | 4.942 | 5.132 | 5.292 | 7.629 | 8.198 | 8.392 | 14.599 |
| 6 | 3.546 | 4.835 | 5.092 | 5.322 | 7.594 | 8.32 | 8.462 | 14.982 |
| RSD(%) | 1.18 | 1.24 | 0.80 | 0.84 | 0.26 | 0.79 | 0.94 | 1.34 |

Table 2. Precision of the peak area of eight standard disaccharides obtained by CZE separation

| Disaccharides | △UA→ GalNAc Na | △UA→ GalNAc-6S$Na_2$ | △UA→ GalNAc-4S$Na_2$ | △UA-2S→ GalNAc$Na_2$ | △UA → GalNAc-4S,6S$Na_3$ | △UA-2S→ GalNAc-6S$Na_3$ | △UA-2S→ GalNAc-4S$Na_3$ | △UA-2S→ GalNAc-4S-6S$Na_4$ |
|---|---|---|---|---|---|---|---|---|
| run | Peak area | | | | | | | |
| 1 | 14235 | 16190 | 13974 | 3355 | 4594 | 4306 | 3999 | 5601 |
| 2 | 14663 | 16439 | 13726 | 3456 | 4478 | 4326 | 3981 | 5682 |
| 3 | 14263 | 15973 | 13783 | 3389 | 4526 | 4402 | 3894 | 5702 |
| 4 | 14829 | 16783 | 13789 | 3383 | 4520 | 4432 | 3893 | 5593 |
| 5 | 14734 | 16538 | 13543 | 3386 | 4493 | 4468 | 3937 | 5671 |
| 6 | 14673 | 16583 | 13625 | 3326 | 4423 | 4431 | 3847 | 5704 |
| RSD(%) | 1.74 | 1.78 | 1.09 | 1.28 | 1.26 | 1.47 | 1.48 | 0.88 |

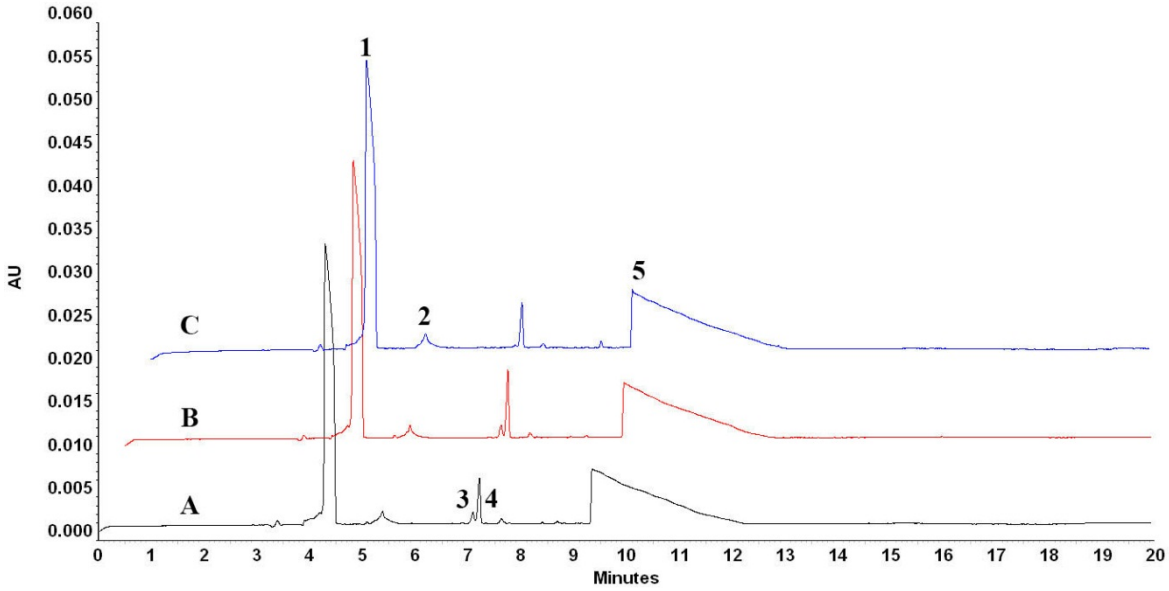


**Fig. 3.** Electrophoretically mediated microanalysis of CS from different sources. (A) cow, (B) pig, (C) shark. Fused silica capillary (total length: 60.2 cm, effective length: 50 cm, i.d. 50 μm). Capillary temperature: 37 ℃. Sample temperature: 4 ℃. Separation buffer: pH 9.5, 25 mM tetraborate buffer. Incubation buffer: pH 8.0, 50 mM Tris-60 mM acetate buffer (0.5 psi×10 s). Voltage: +20 kV. Injection: CS solution (0.5 psi×5 s), enzyme solution (0.5 psi×5 s). Voltage switch sequence: -1 kV/+1 kV/-1 kV/+1 kV, each for 6 s. In capillary incubation time: 8min. Detection wavelength: 200nm. Peaks identification: (1) solvent peak of incubation buffer, (2) enzyme, (3) ΔDi-6S, (4) ΔDi-4S, (5) solvent peak of incubation buffer.

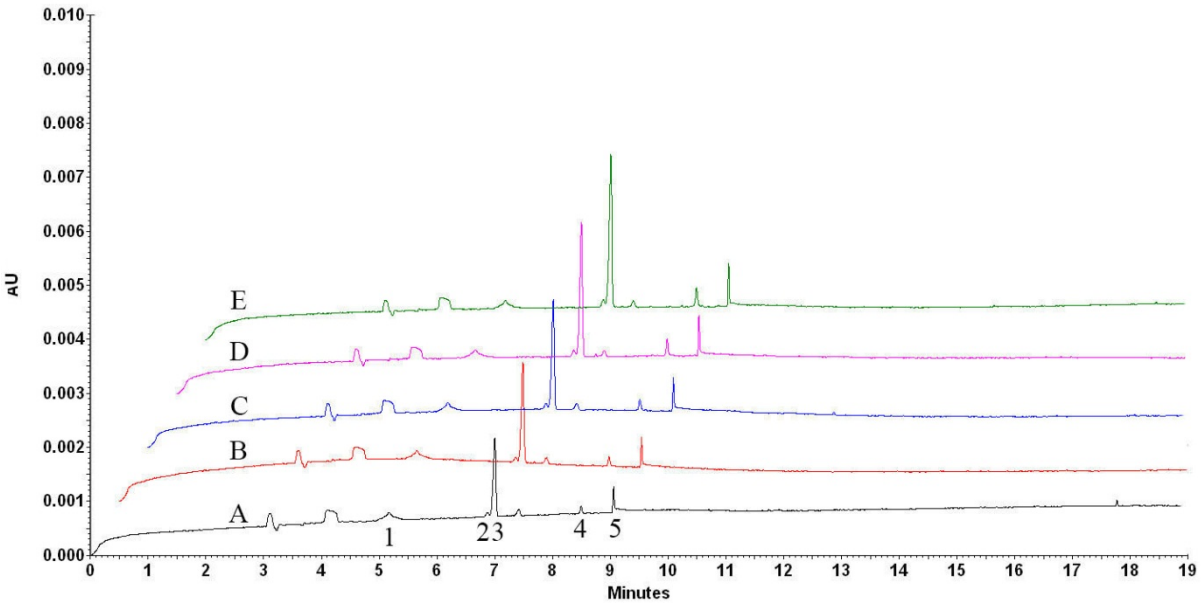


**Fig. 4.** Electrophoretically mediated microanalysis of CS of different concentrations. (A) 300 μg/mL, (B) 400 μg/mL, (C) 500 μg/mL, (D) 600 μg/mL, (E) 700 μg/mL. Detection wavelength: 232nm. Peaks identification: (1) enzyme, (2) ΔDi-6S, (3) ΔDi-4S, (4) ΔDi-UA2S, (5) solvent peak of incubation buffer. Other conditions were as in Fig. 3

Table 3. Precision of the migration time of three disaccharides obtained by EMMA method

| Disaccharides | △UA→GalNAc-6S$Na_2$ | △UA→GalNAc-4S$Na_2$ | △UA → GalNAc-4S,6S$Na_3$ |
|---|---|---|---|
| run | | time(min) | |
| 1 | 7.088 | 7.208 | 8.683 |
| 2 | 7.134 | 7.293 | 8.572 |
| 3 | 7.201 | 7.196 | 8.672 |
| 4 | 6.998 | 7.261 | 8.695 |
| 5 | 7.042 | 7.294 | 8.892 |
| 6 | 7.067 | 7.277 | 8.693 |
| RSD(%) | 1.01 | 0.59 | 1.20 |

Table 4. Precision of the peak area of three disaccharides obtained by EMMA method

| Disaccharides | △UA→GalNAc-6S$Na_2$ | △UA→GalNAc-4S$Na_2$ | △UA → GalNAc-4S,6S$Na_3$ |
|---|---|---|---|
| run | | peak area | |
| 1 | 2959 | 2890 | 97 |
| 2 | 2893 | 2886 | 98 |
| 3 | 3015 | 2796 | 101 |
| 4 | 2967 | 2885 | 99 |
| 5 | 2899 | 2936 | 96 |
| 6 | 3009 | 2894 | 97 |
| RSD(%) | 1.77 | 1.59 | 1.83 |