Electronic Supplementary Material (ESI) for Analyst


# Supporting information

# An Enhanced Capillary Electrophoresis Method for Characterizing Natural Organic Matter

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Current vs. pH

| | pH | 3.13 | 2.95 | 2.54 | 2.33 | 2.15 | 1.95 | 1.75 |
|---|---|---|---|---|---|---|---|---|
| [M] | | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| I* | | 0.20 | 0.20 | 0.21 | 0.21 | 0.21 | 0.22 | 0.23 |
| μA | | -89 | -87 | -98 | -110 | -119 | -144 | -167 |
| s.d. | | ± -3 | ± -1 | ± -3 | ± -1 | ± -2 | ± -1 | ± -1 |

Current vs. buffer concentration

| [M] | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 |
|---|---|---|---|---|---|
| EOF | 8.1 | 5.0 | nd | 3.0 | nd |
| s.d. | ± 0.1 | ± 0.1 | nd | ± 0.1 | nd |
| I* | 0.05 | 0.11 | 0.16 | 0.21 | 0.26 |
| μA | -38 | -64 | -77 | -110 | -146 |
| s.d. | ± -1 | ± -1 | ± -1 | ± -1 | ± -2 |

**Table S1**. Summary of the effect of pH and buffer concentration on capillary,current, and EOF. [M]: concentration of sodium phosphate buffer (mol/L); I: ionic strength (mol/kg) (http://www.chembuddy.com); μA: capillary current ($10^{-6}$ ampere); and EOF ($10^{-5}$ $cm^2$/Vs. Measurements are 20°C, 20 kV. Values are the average of three runs and the error expressed as standard deviation (s.d.). EOF of the sodium borate buffer is 4 x 10-4 cm2/Vs.
*Ionic strength calculations are not accurate at buffer concentrations greater tthan 0.1M.

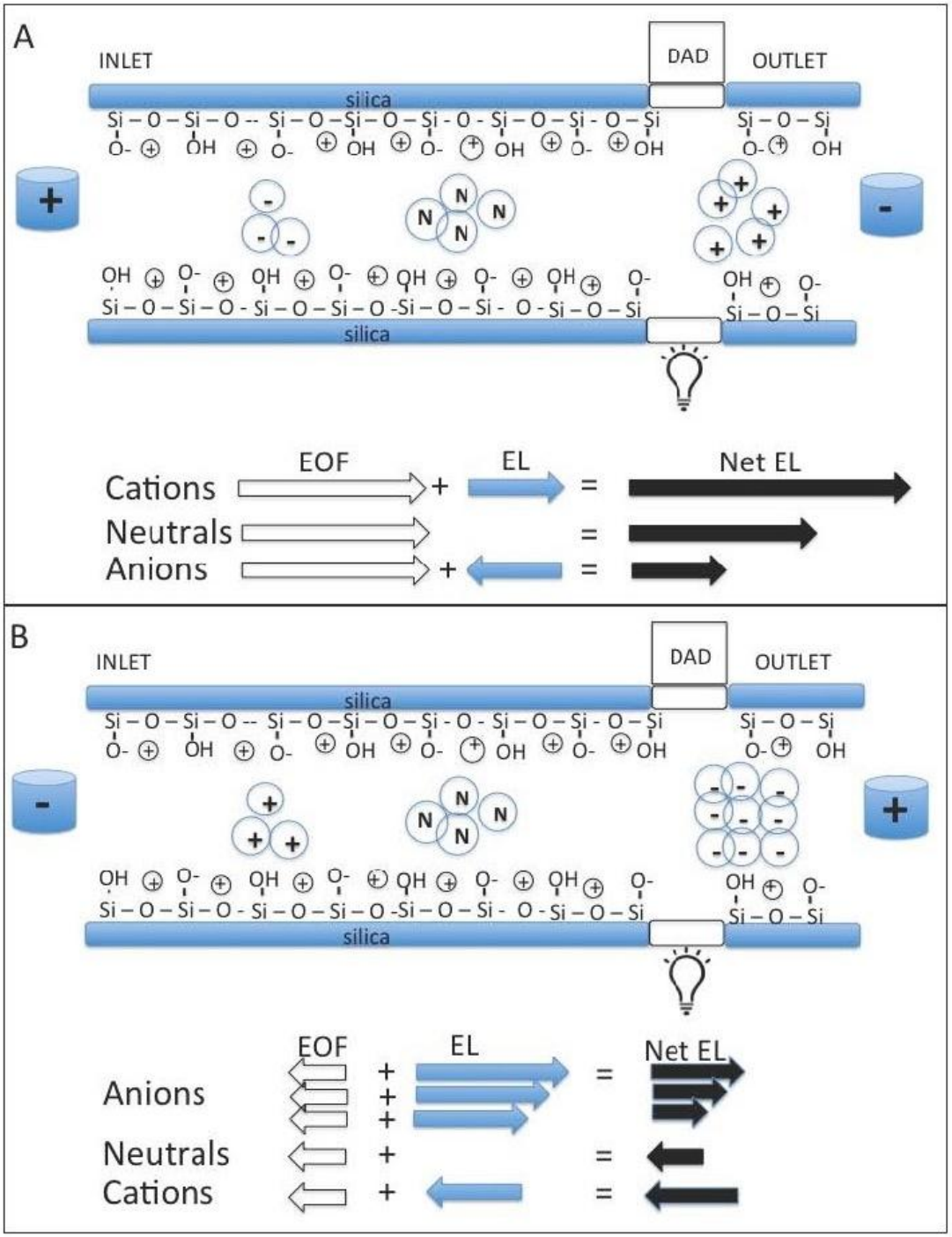


**Fig. S1** A schematic demonstrating the principles of (A) Conventional CE at high pH with a dominant EOF (B) Counterbalance CE (polarity reversal) and a reduced EOF (low pH). The counterbalance conditions result in a longer residence for the analytes in the capillary.

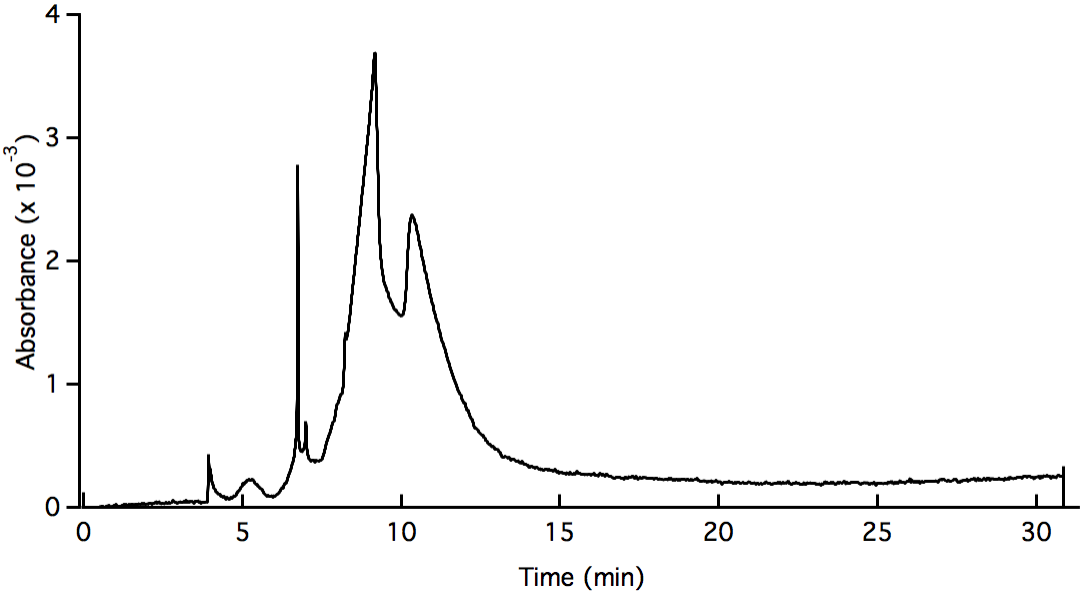


**Figure S2.** Electropherogram using conventional CE of SRNOM (10 mg/ml) in 0.1 M sodium borate buffer (pH = 8.3), normal electrode polarity. The EOF is 4 x $10^{-4}$ $cm^2$/Vs. Absorbance measured 214 nm.

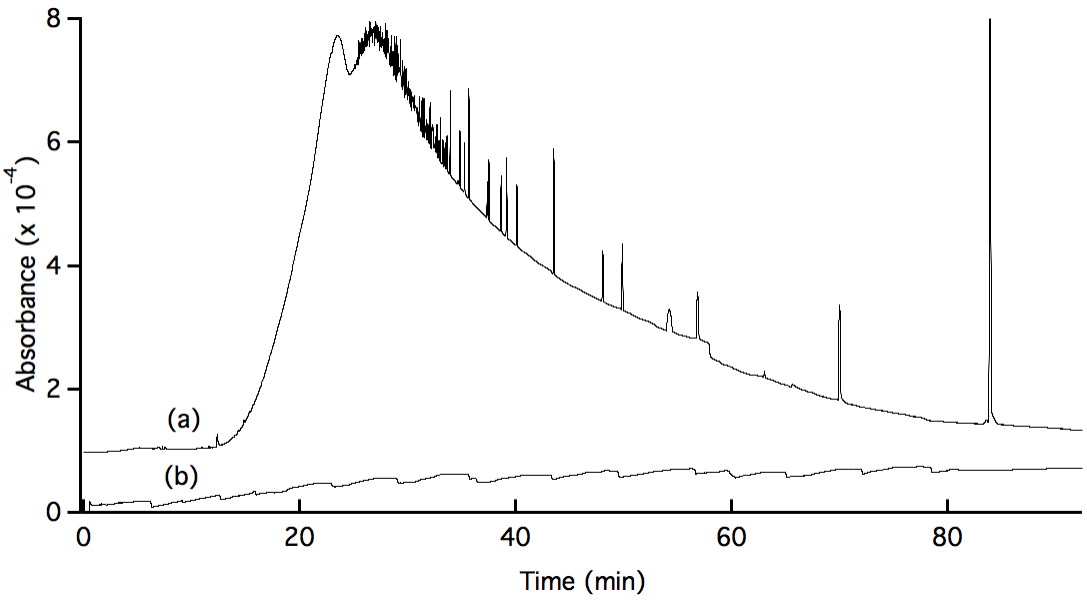


**Figure S3**. Electropherograms of SRNOM using (a) counterbalance CE (reverse polarity, -20 kV) and (b) conventional CE (normal polarity 20). Background electrolyte is 0.2M phosphate buffer, pH = 2.33, 20$^{o}$C. Absorbance measured at 214 nm.

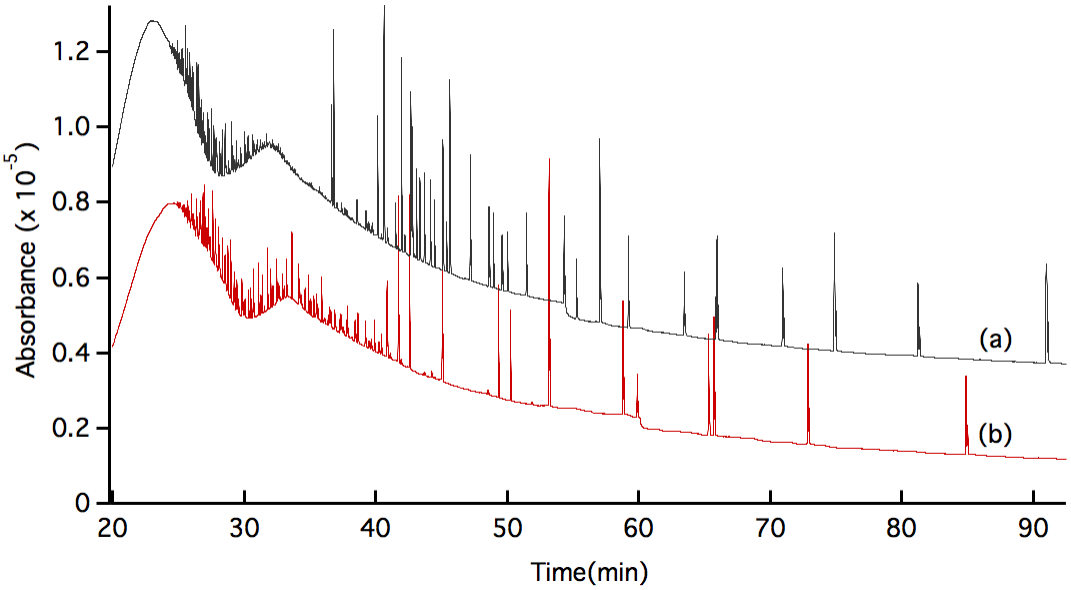


**Figure S4.** Electropherograms demonstrating the effect of organic solvent on peak resolution: (a) without and (b) with 5% acetonitrile. Electropherograms are offset on Y-axis. The X-axis is expanded to demonstrate increased resolution. Run conditions: 0.2 M phosphate buffer (pH = 2.33, - 20KV, 20$^0$C). Absorbance measured at 214 nm.

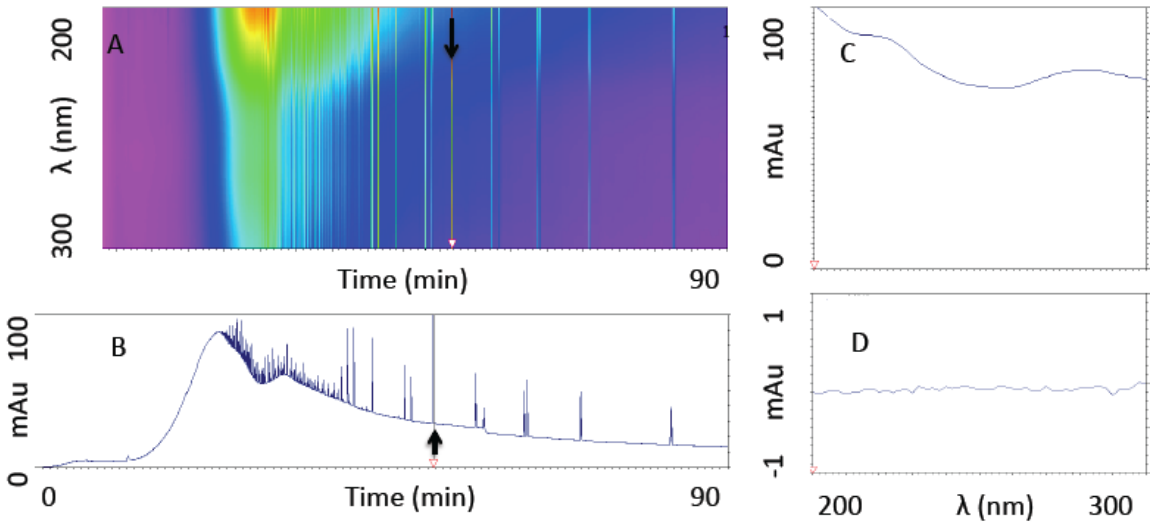


**Fig. S5.** DAD composite of SRNOM electropherogram: **A**. contour plot (200 – 300 nm), **B.** electropherogram (0 – 90 min.), **C.** spectrum at 50 min (designated by arrow in contour and electropherogram), and **D**. zero absorbance of an air-filled capillary (insert).

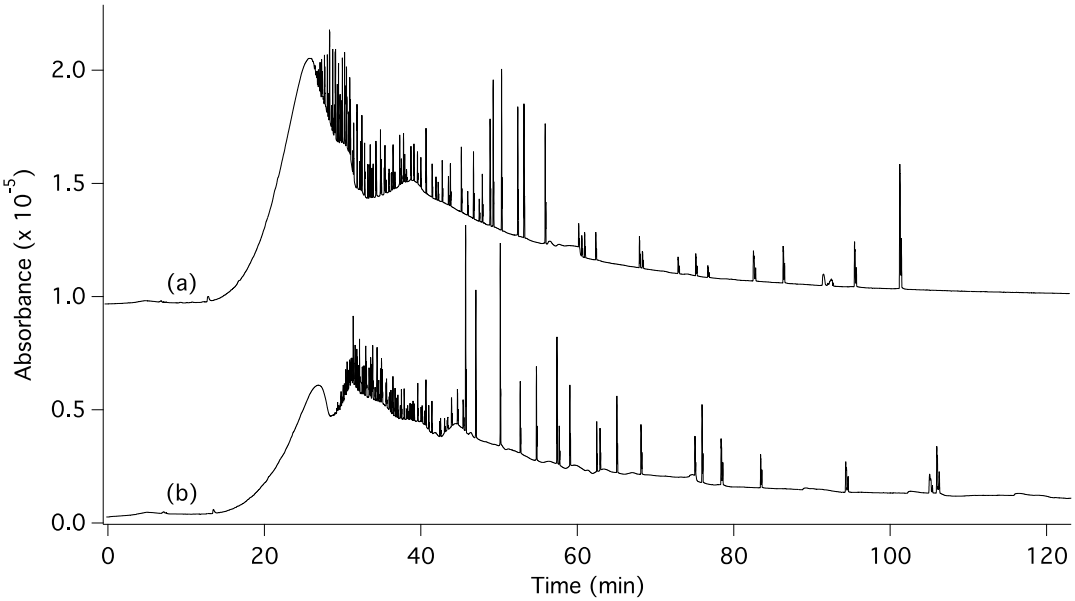


**Figure S6.** Electropherograms of SRNOM before optimization (a) 15 cm capillary (b) 50 cm capillary. Electropherograms are offset on Y-axis. Run conditions: 0.2 M phosphate buffer (pH = 2.33, - 20KV, 20$^0$C). Absorbance measured at 214 nm.

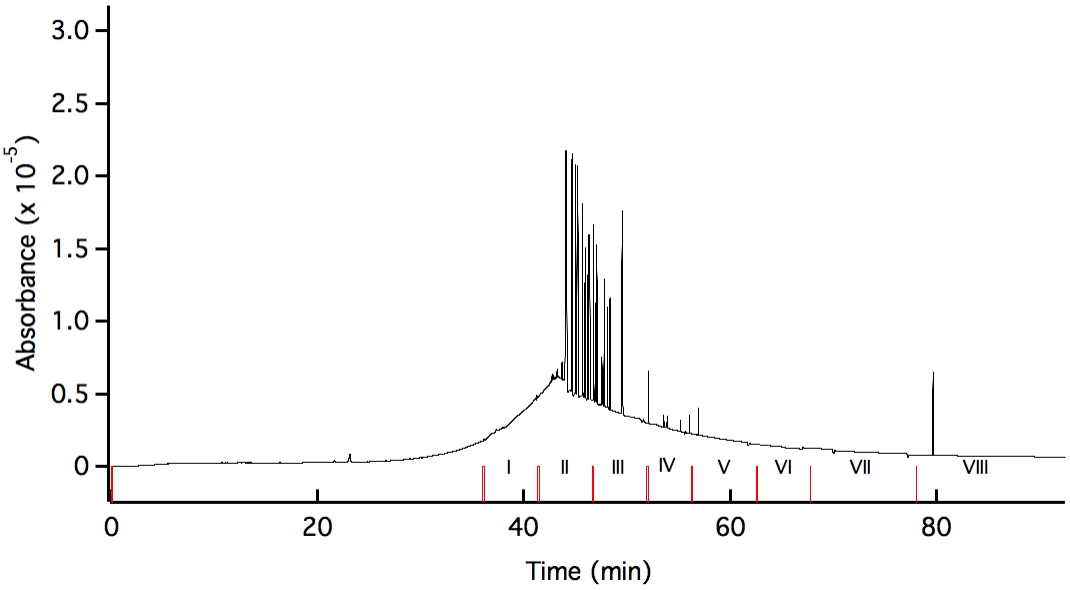


**Figure S7.** Electropherogram of SRNOM (50 mg/ml) with fraction collection (-10KV, 20$^0$C). The current drop to zero μA (red overlay) registers each fraction collection event. Absorbance is measured at 214 nm.

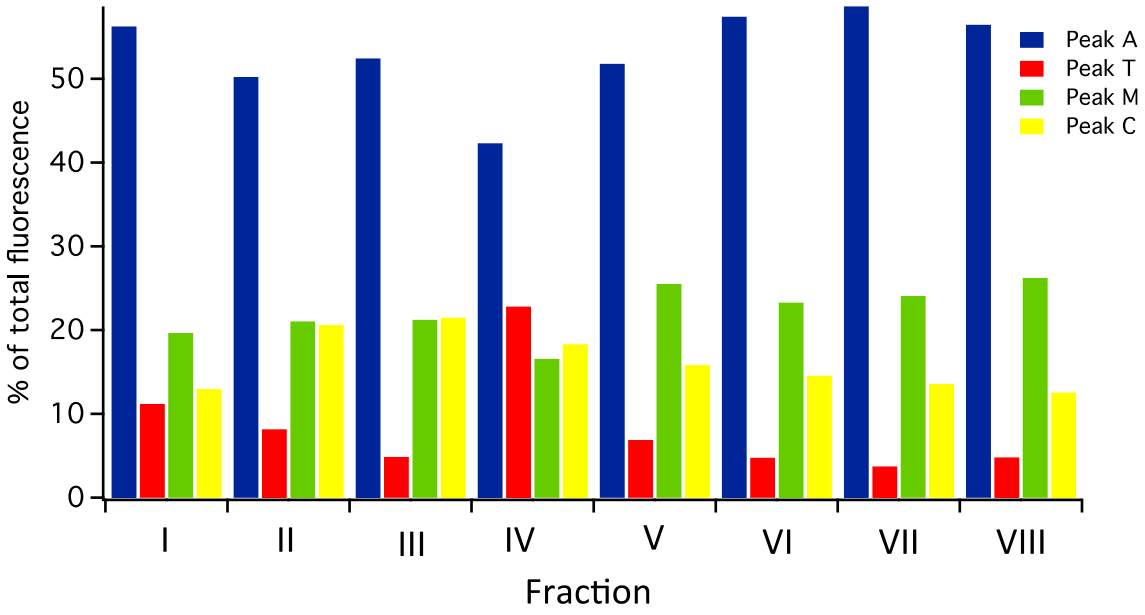


**Figure S8.** Duplicate run of CE fractionated SRNOM. Factions from 24 consecutive runs were pooled. Histograms represent the relative fluorescence (fraction of total fluorescence) of the EMMs peaks (A, T, M, and C).

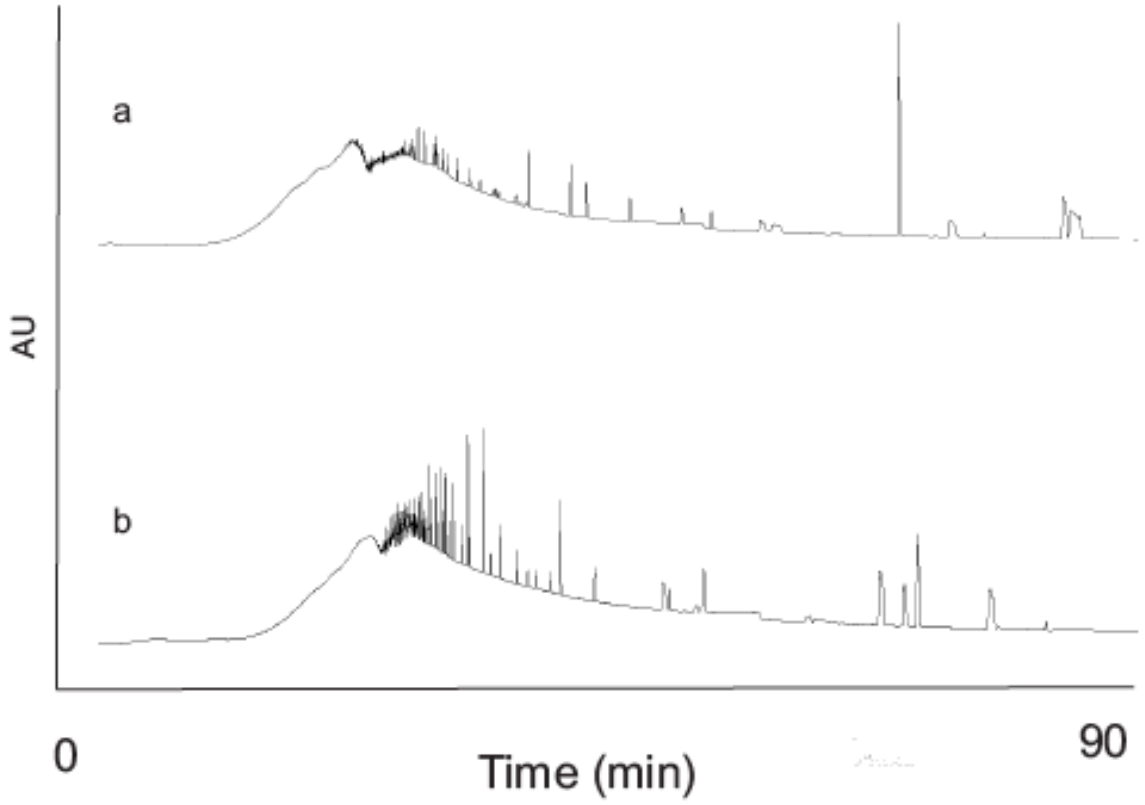


**Figure S9.** Electropherograms demonstrating the effect of ramp time on the peak resolution: (a) 0.17 min (b) 5 min. Run conditions: 0.2 M phosphate buffer (pH = 2.33, - 20KV, $20^0$C). Absorbance measured at 214 nm.

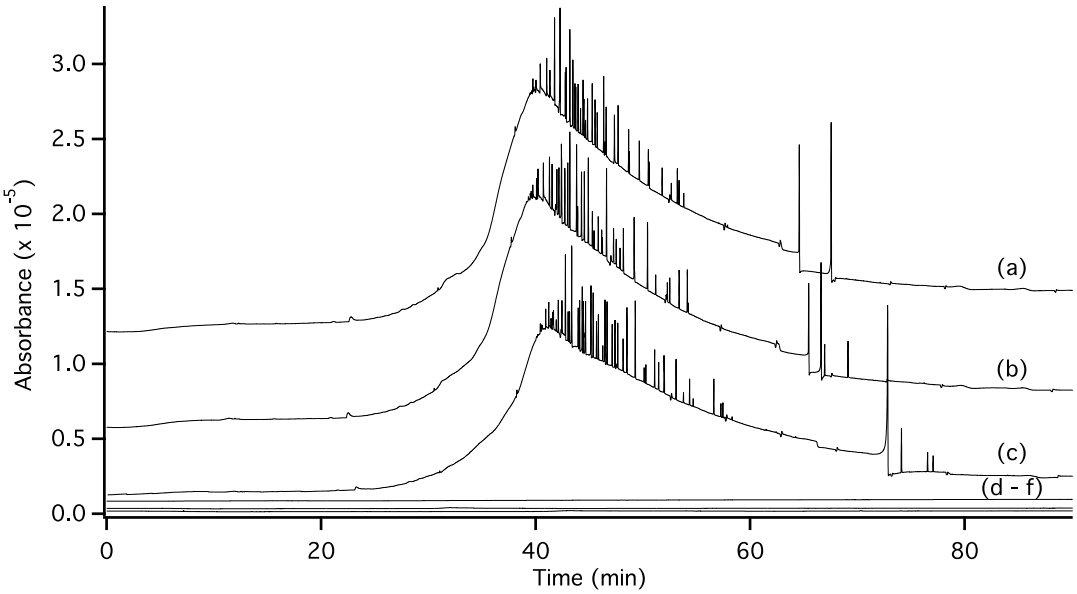


**Figure S10.** Electropherograms demonstrating variation in electrophoretic mobility for a sequence of 24 fraction collection runs: **(a)** run #1, **(b)** run #8, **(c)** run #24, **(d –f)** blank runs (buffer only) spaced after every six runs. Running conditions: SRNOM (100 mg/ml, -10kV, 20$^0$C). Average deviation in migration times = 2.3 min for the peaks between 60 – 80 min. The peaks in the time range 40 – 60 min have a mobility shift 0.1 % to 0.6 % of the 90 min run time. The range mobility shift as percent of total run time is 2.3 – 6.3 %.