† Electronic Supplementary Information (ESI)

Cation/anion-exchange mode switching chromatography utilizing pH-responsive mixed charge polymer-modified silica beads

*Taisei Kaku, Koichi Deura, Tomoka Yoshii, Daniel Citterio, and Yuki Hiruta**

† Department of Applied Chemistry, Faculty of Science and Technology, Keio University

3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

Corresponding Author

**E-mail: hiruta@applc.keio.ac.jp*

Table S1. Characterization of pH-responsive mixed-charge hydrogel modification to aminopropyl silica beads utilizing elemental analysis. Elemental composition was determined by CHN elemental analysis. The grafted amount of initiator and the polymers on the silica beads were estimated using the carbon composition.

Code	Elemental composition (%)			Immobilized initiator ($\mu mol/m^2$)	Grafted polymer (mg/m^2)
	C	H	N		
APS	3.16±0.01	0.92±0.01	1.16±0.01		
V501S	7.17±0.02	1.25±0.01	2.65±0.01	1.51	
ACSuc	16.16±0.01	2.49±0.01	1.98±0.01		1.05
ACSucEE	14.51±0.02	2.27±0.01	1.94±0.01		0.78
ACCHexEE	14.60±0.01	2.28±0.01	1.94±0.01		0.76

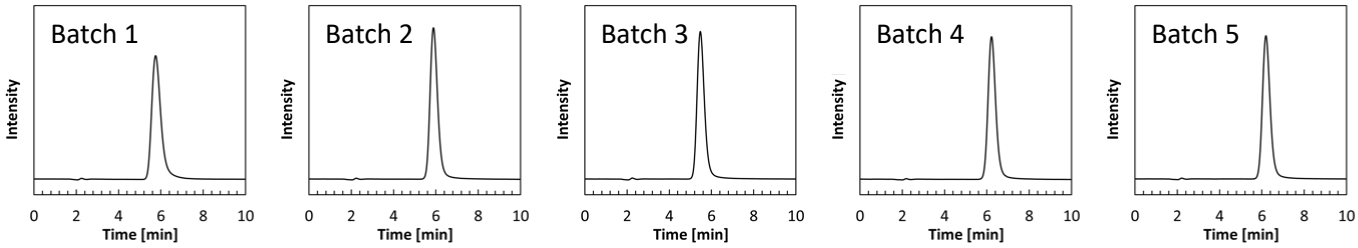

Fig. S1 Batch-to batch reproducibility of ACSuc columns tested with naphthalene. Analytical conditions: mobile phase: 100 mM citric acid buffer (pH 7)/methanol (70/30, v/v); column temperature: 40 °C; flow rate: 0.2 mL/min; concentration: 0.1 mg/mL; injection volume: 5 µL.

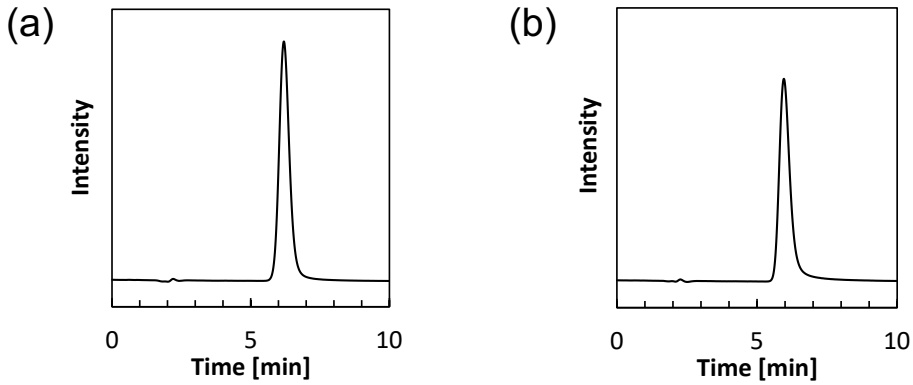

Fig. S2 Stability test for the ACSuc column with napthalene (a) Before and (b) after purging 5000 column volumes of mobile phase. Analytical conditions: mobile phase: 100 mM citric acid buffer (pH 7)/methanol (70/30, v/v); column temperature: 40 °C; flow rate: 0.2 mL/min; concentration: 0.1 mg/mL; injection volume: 5 µL.

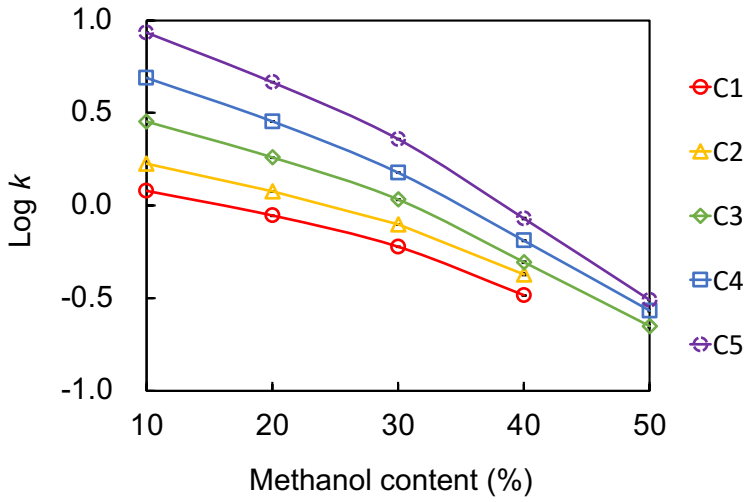

Fig. S3 Retention factors of C1-C5 alkylbenzenes depending on methanol content in the mobile phase with ACSuc column. Analytical conditions: mobile phase: 100 mM citric acid buffer (pH 7)/methanol; column temperature: 40 °C; flow rate: 0.2 mL/min; concentration: 0.1 mg/mL; injection volume: 5 µL.

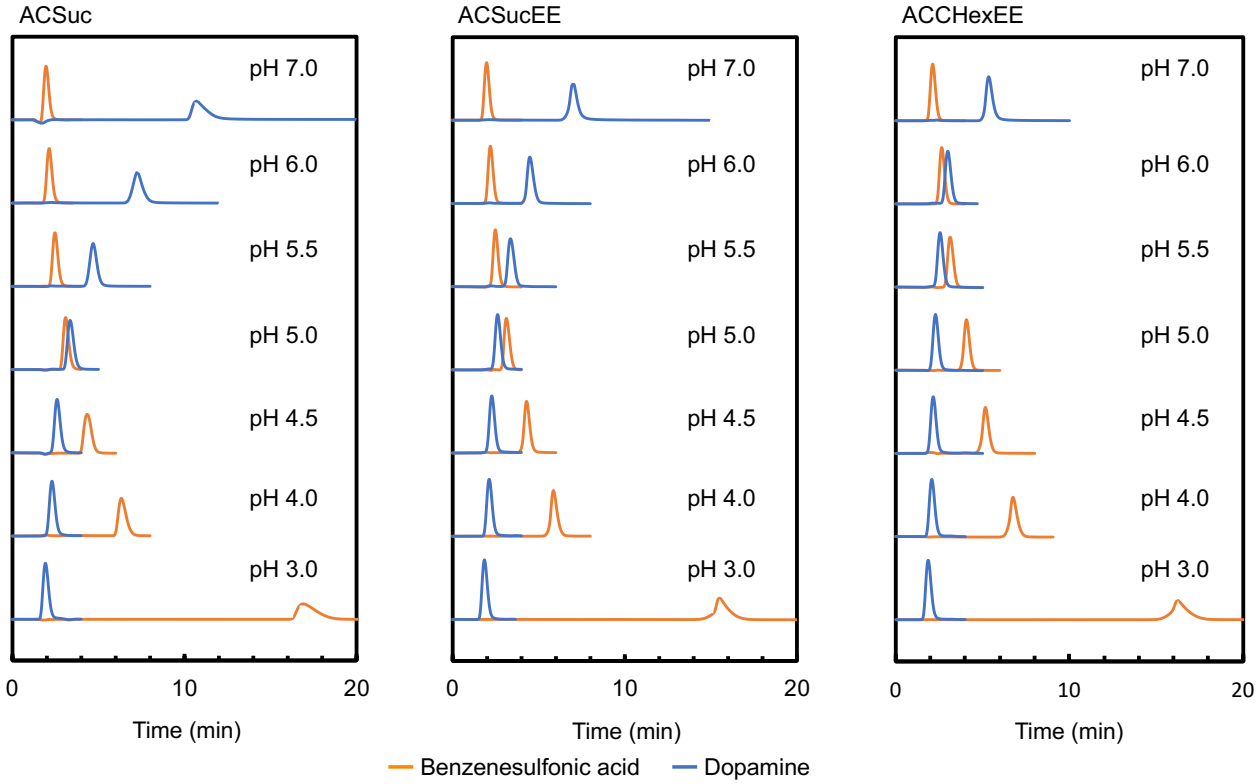

Fig. S4 Chromatograms of benzenesulfonic acid and dopamine with ACSuc, ACSucEE, and ACCHexEE; Analytical conditions: mobile phase: 100 mM citric acid buffers; column temperature: 40 °C; flow rate: 0.2 mL/min; concentration: 1 mg/mL; injection volume: 5 µL.

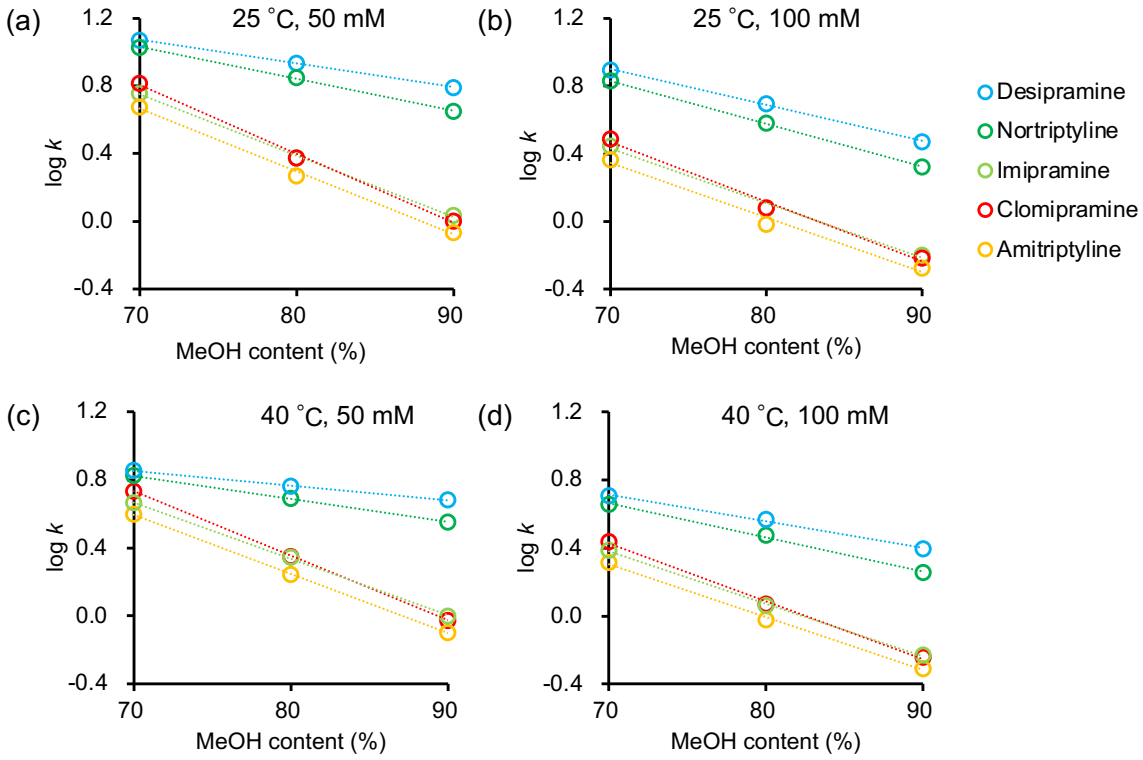

Fig. S5 Retention factors of five antidepressants on an ACCHexEE column at each ionic strength of mobile phase, and temperature. Analytical condition: mobile phase: (a, c) 50 mM or (b, d) 100 mM citrate buffer (pH 7)/methanol; column temperature: (a, b) 25 °C or (c, d) 40 °C; flow rate: 0.2 mL/min; concentration: 0.1 mg/mL, 10 µL injection.

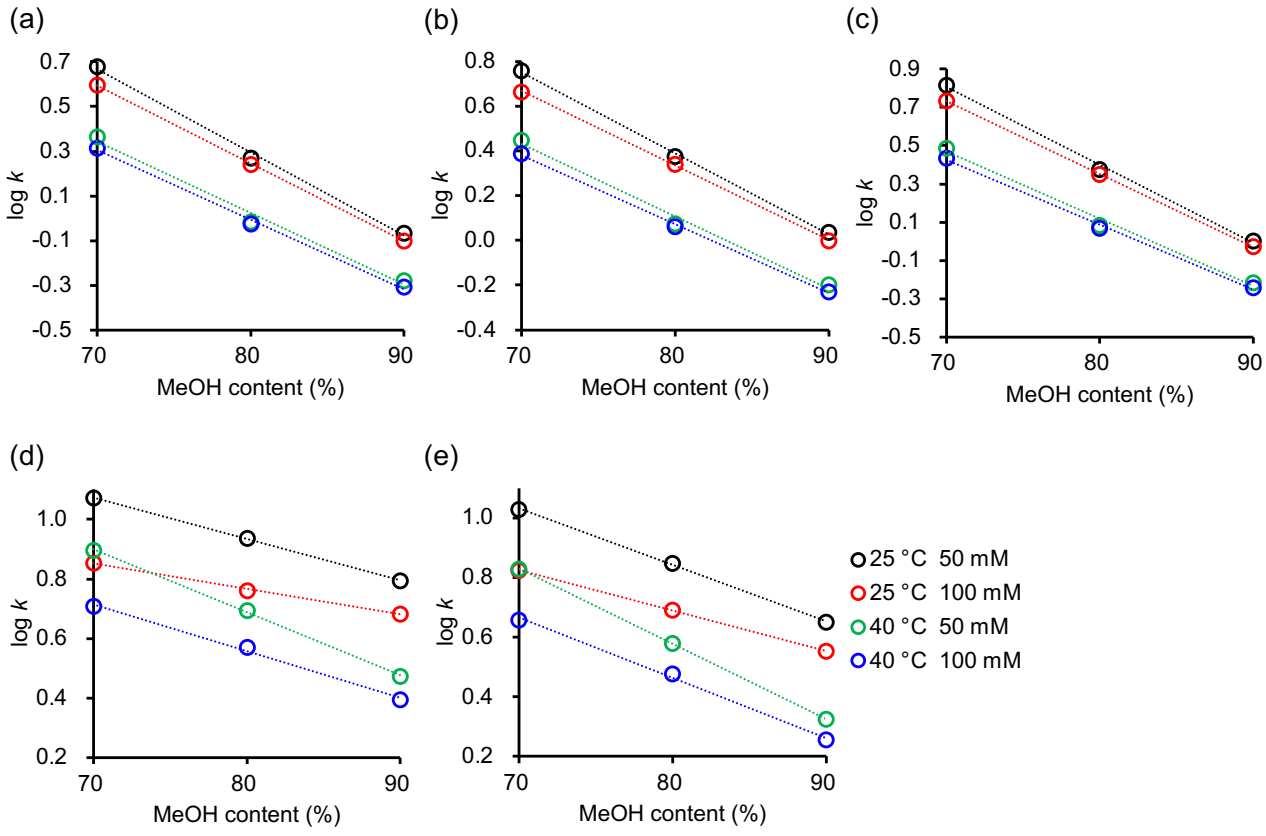

Fig. S6 Comparison of retention factors of (a) amitriptyline, (b) imipramine, (c) clomipramine, (d) nortriptyline, (e) desipramine at different column temperatures and ionic strength of mobile phase.

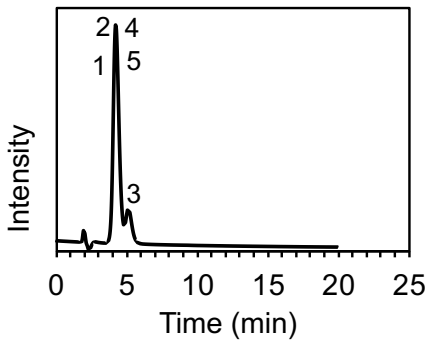

Fig. S7 Chromatogram of mixed sample of 1. amitriptyline, 2. imipramine, 3. clomipramine, 4. nortriptyline, and 5. desipramine on an ACCHexEE column. Analytical condition: mobile phase: 50 mM citrate buffer (pH 5)/methanol (30/70, v/v); column temperature: 25 °C; flow rate: 0.2 mL/min; concentration: 0.1 mg/mL, 10 μL injection.

Table S2. Retention factors of five antidepressants on an ACCHexEE column. Analytical condition: mobile phase: 50 mM citrate buffer (pH 5)/methanol (30/70, v/v); flow rate: 0.2 mL/min; concentration: 0.1 mg/mL, 10 μL injection.

	pH 7	pH 5
1. Amitriptyline	0.679	0.225
2. Imipramine	0.759	0.232
3. Clomipramine	0.817	0.302
4. Nortriptyline	1.03	0.225
5. Desipramine	1.07	0.219