

1 **Reversed-phase/hydrophilic bifunctional interaction mixed-mode**
2 **monolithic column with biphenyl and quaternary ammonium**
3 **stationary phases for capillary electrochromatography**

4

5 Zhenkun Mao^{1,2}, Changjun Hu¹, Zhentao Li¹, Zilin Chen^{1,2*}

6 1. Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry
7 of Education, and Wuhan University School of Pharmaceutical Sciences,
8 Wuhan, 430071, China

9 2. State Key Laboratory of Transducer Technology, Chinese Academy of
10 Sciences, Beijing 10080, China

11

12

13

14

15

16 **Corresponding author**

17 Dr. Zilin Chen

18 Luojia Chair Professor

19 Vice Dean and Institute Director

20 School of Pharmaceutical Sciences,

21 Wuhan University

22 Wuhan, 430071

23 CHINA

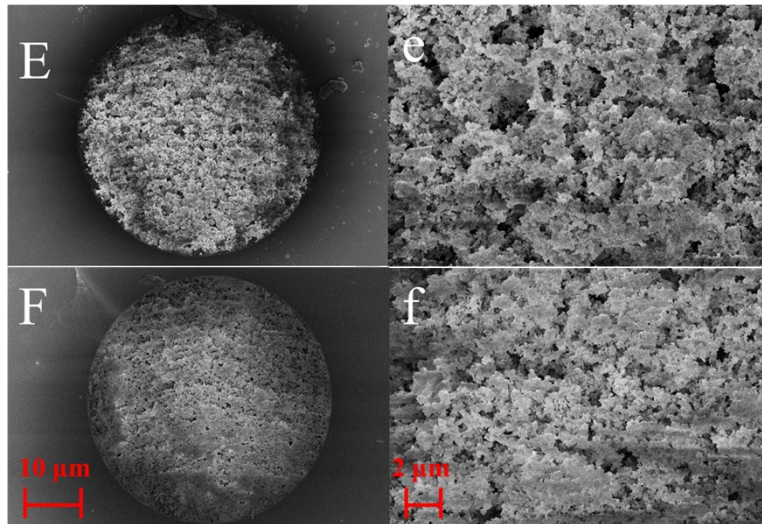
24 Phone: 86-27-68759893

25 Fax: 86-27-68759850

26 Email: chenzl@whu.edu.cn

27

28



29

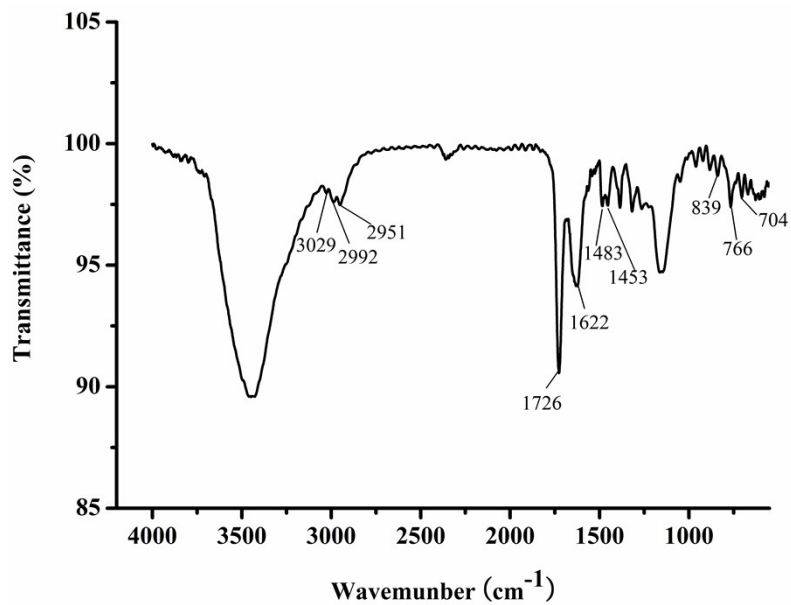
30 **Fig. S1.** Characterization of SEM for the monoliths (column No. 7: E, 750 ×; e, 3000

31 ×. Column No. 8: F, 750 ×; f, 3000 ×).

32

33

34

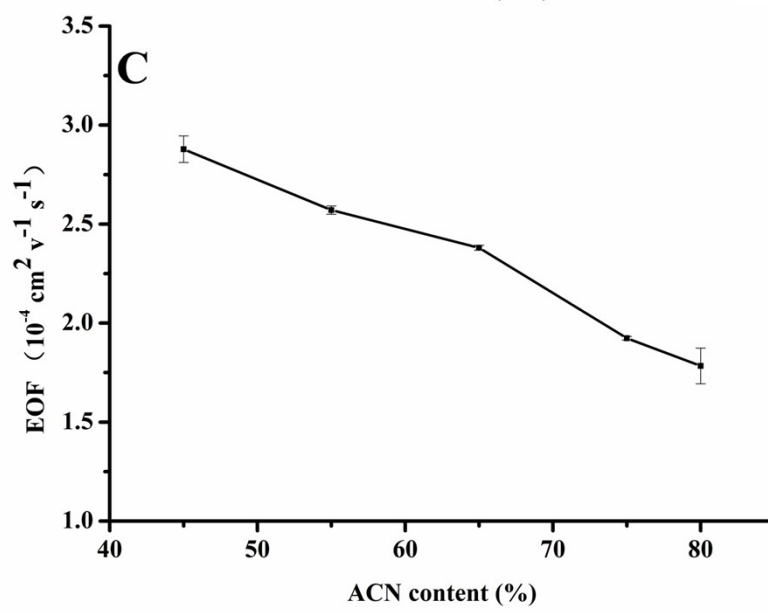
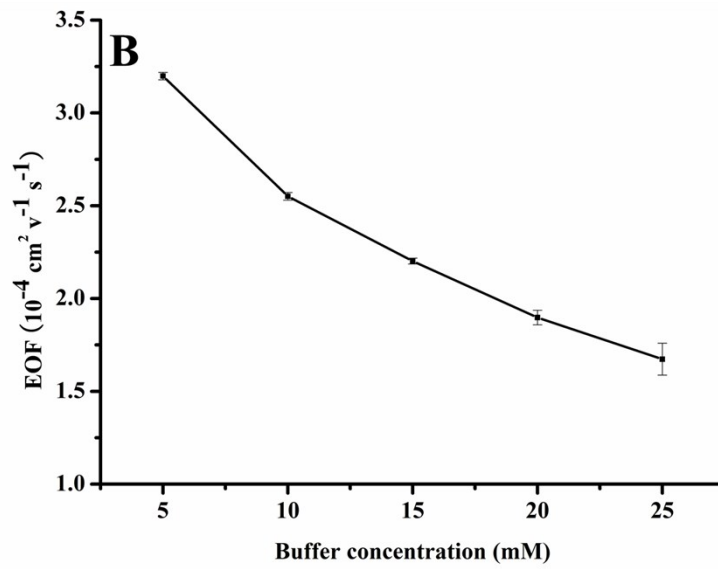
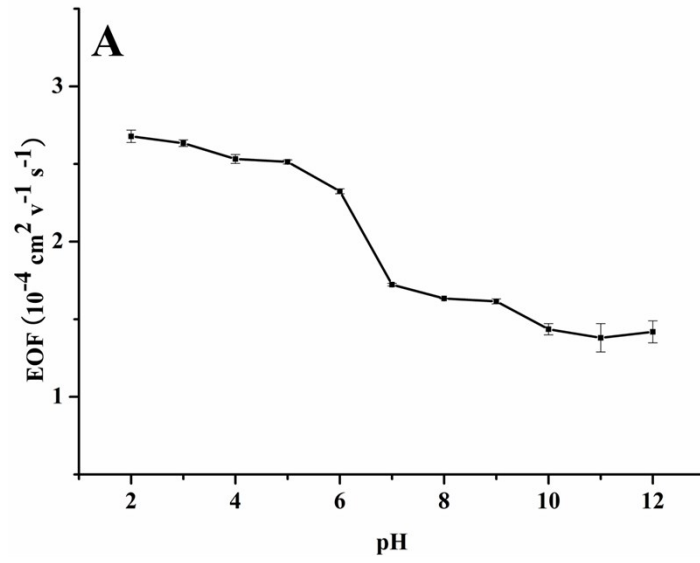


35

36

37 **Fig. S2.** FT-IR spectra of the poly(VBP-*co*-EDMA-*co*-VBTA) monolith (column No.

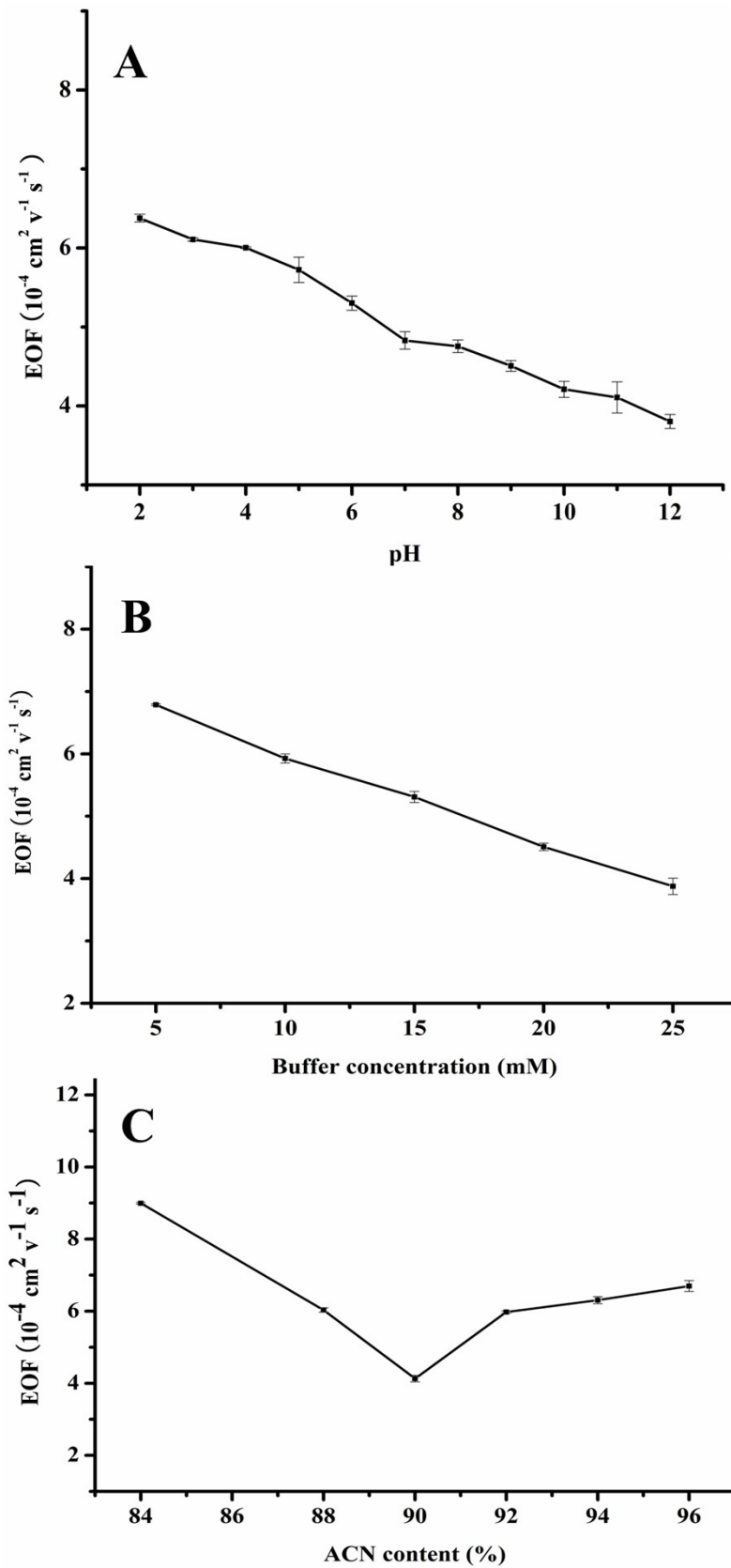
38 5).



39

40

41 **Fig. S3.** Influence of pH (A), buffer concentration (B), ACN content (C) in RP-mode
42 to the EOF mobility on monolithic column No. 5. Experimental conditions: mobile
43 phase, (A) 10 mM phosphate buffer (pH 2.0–12.0) with 55% ACN, (B) pH 4.0
44 phosphate buffer (5.0–25.0 mM) with 55% ACN, (C) phosphate buffer (10 mM, pH
45 4.0) with different content ACN; applied voltage, – 20 kV; electrokinetic injection, –
46 5 kV × 5 s; detection wavelength, 214 nm; EOF marker, DMF.

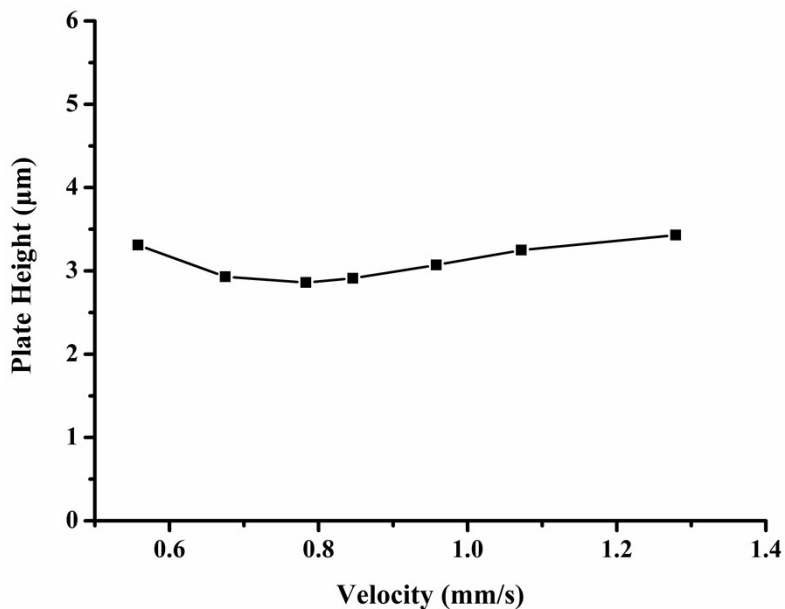


47

48 **Fig. S4.** Influence of pH (A), buffer concentration (B), ACN content in HILIC-mode
 49 (C) to the EOF mobility on monolithic column No. 5. Experimental conditions:

50 mobile phase, (A) 10 mM phosphate buffer (pH 2.0–12.0) with 92% ACN, (B) pH 4.0
51 phosphate buffer (5.0–25.0 mM) with 92% ACN, (C) phosphate buffer (10 mM, pH
52 4.0) with different content ACN; applied voltage, – 20 kV; electrokinetic injection, –
53 5 kV × 5 s; detection wavelength, 214 nm; EOF marker, acetophenone.

54



55

56

57 **Fig. S5.** Effect of linear velocity of mobile phase on the plate height of the monolithic
58 column (No. 5). Experimental conditions: mobile phase, 55% ACN in pH 4.0 10 mM
59 phosphate buffer; applied voltage, from – 12 kV to – 25 kV; electrokinetic injection,
60 – 5 kV × 5 s; detection wavelength, 214 nm. Marker: benzene.

Table S1Compositions of the polymerization mixtures for the poly(VBP-*co*-EDMA-*co*-VBTA) monoliths.

Column	Monomers/porogens (wt%)	Monomers			Porogens		Backpressure (MPa)	Permeability (10 ⁻¹⁴ m ²)
		4-Vinylbiphenyl (wt%)	VBTA (wt%)	EDMA (wt%)	Cyclohexanol (wt%)	Dodecanol (wt%)		
1	15:85	0	6	9	42.5	42.5	0.7	3.28
2	15:85	2.2	3.8	9	42.5	42.5	0.3	6.97
3	15:85	3	3	9	42.5	42.5	0.5	4.18
4	15:85	3.8	2.2	9	46.8	38.2	1.2	1.74
5	15:85	3.8	2.2	9	42.5	42.5	0.8	2.61
6	15:85	3.8	2.2	9	38.2	46.8	0.5	4.18
7	15:85	4.6	1.4	9	42.5	42.5	2.2	0.95
8	20:80	5	3	12	40	40	Blocked	–

“–” is no detection.

Table S2

Intra-day and inter-day (n = 5), column-to-column and batch-to-batch (n = 3) RSDs of the monolithic column (No. 5) for separation of five alkylbenzenes under RPLC mode. The experimental conditions are same as Fig. 3.

Analytes	Time (RSDs%)			
	Intra-day (n = 5)	Inter-day (n = 5)	Column-to-column (n = 3)	Batch-to-batch (n = 3)
DMF	0.92	1.23	1.86	1.16
Benzene	1.76	2.18	2.29	1.85
Toluene	1.82	2.26	2.93	2.72
Ethylbenzene	1.97	2.87	3.76	3.29
Propylbenzene	2.87	3.92	4.13	3.81
Butylbenzene	3.79	4.68	4.66	4.53

Table S3

Intra-day and inter-day (n = 5), column-to-column and batch-to-batch (n = 3) RSDs of the monolithic column (No. 5) for separation of 6-hydroxypurine and 2,6-dihydroxypurine under HILIC mode. The experimental conditions are same as Fig. 8.

Analytes	Time (RSDs%)			
	Intra-day (n = 5)	Inter-day (n = 5)	Column-to-column (n = 3)	Batch-to-batch (n = 3)
Acetophenone	1.02	0.98	1.39	1.13
6-Hydroxypurine	1.28	0.93	1.89	3.61
2,6-Dihydroxypurine	2.71	2.02	3.36	4.06

Table S4

Comparison of different mixed-mode monolithic columns.

Stationary phases	Mixed-mode	Analytical method	Analytes	Elution time (min)	column efficiency (N/m)	Ref.
Poly(<i>p</i> -MAPHA- <i>co</i> -PETA)	RPLC/HILIC/IEC	cLC	PAHs, nucleosides, basic compounds	25	0.76×10^5	[7]
Poly(DASP- <i>co</i> -PETA)	RPLC/HILIC	cLC	Acidic and basic compounds, alkylbenzenes	25	0.97×10^5	[8]
Sil-G4-BDDE-DA	RPLC/HILIC/IEC	LC	Alkylbenzenes, PAHs, nucleobases, nucleosides	120	–	[9]
Long-alkyl-chain-based hybrid monoliths	RPLC/IEC	CEC	Alkylbenzenes, aromatic amines	12	1.15×10^5	[11]
Quinine-modified poly(GMA- <i>co</i> -EDMA) monoliths	RPLC/IEC	CEC	Alkylbenzenes, PAHs acidic compounds, excitants	17	1.90×10^5	[15]
poly(VBP- <i>co</i> -EDMA- <i>co</i> - VBTA) monoliths	RPLC/HILIC	CEC	Vanillin substances, neutral and alkaline compounds	9	3.49×10^5	This work

“–” No data.