Electronic Supplementary Information

Phosphatidic acid-functionalized monolithic stationary phase for reversed-phase/cation-exchange mixed mode chromatography

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.

Figure

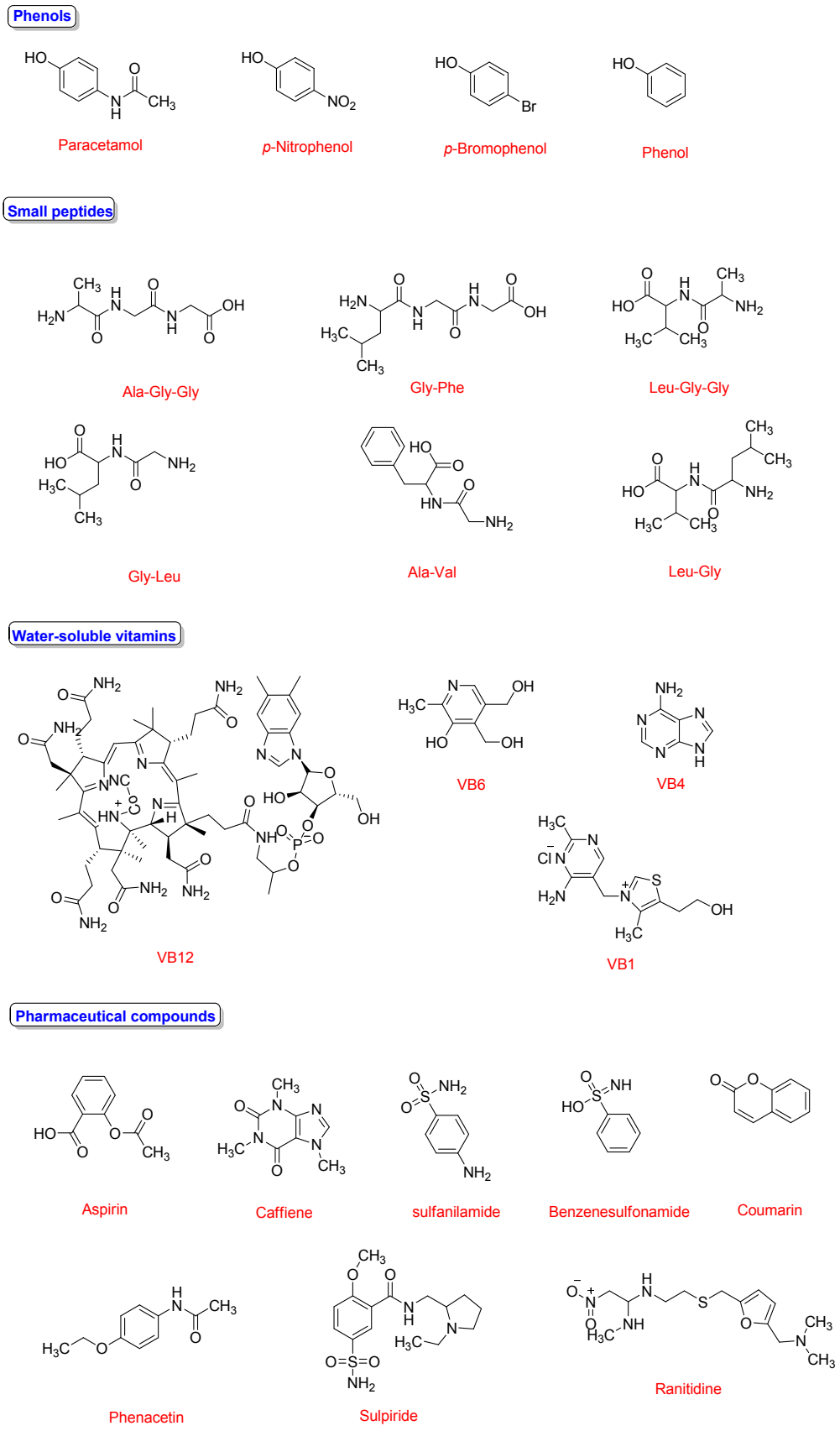

Fig. S1. Chemical structures of test compounds employed in the present study.

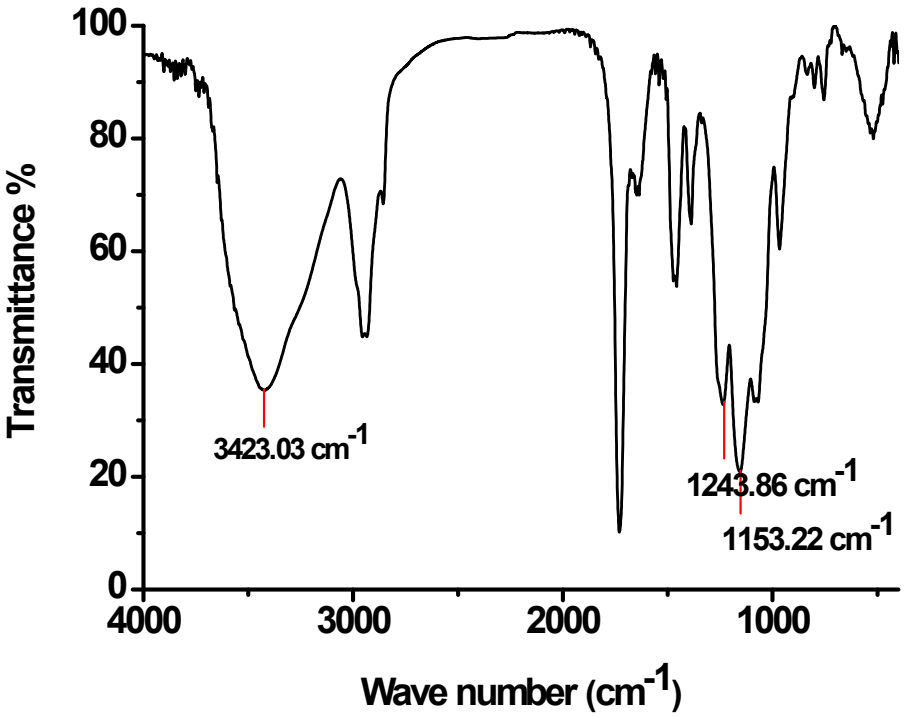

Fig. S2. Fourier transform infrared spectra of poly(MDPA-*co*-EDMA) monolithic stationary phases.

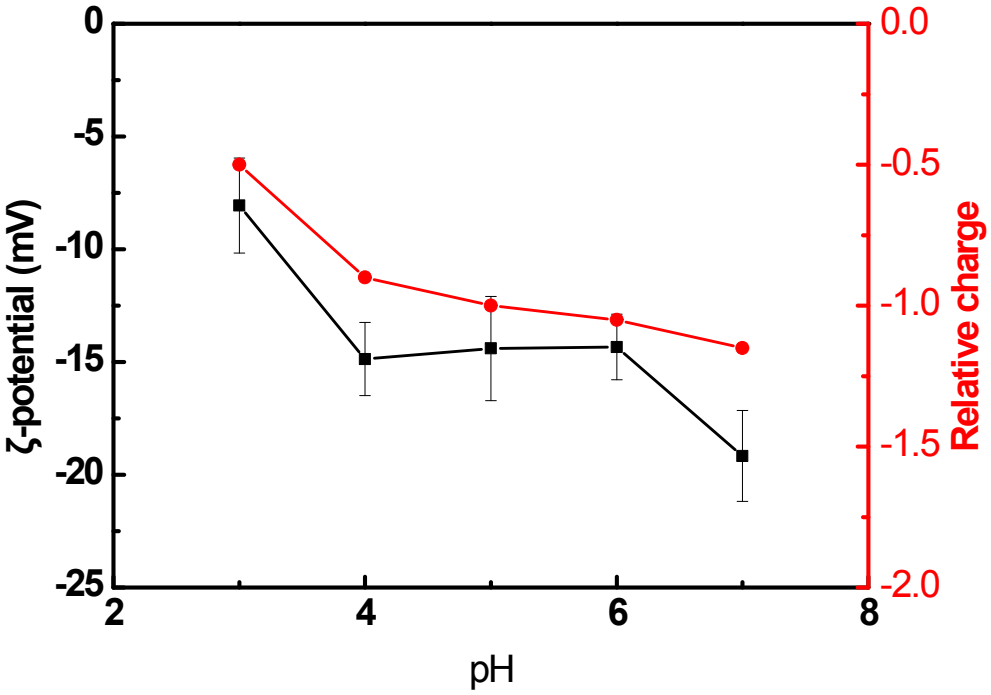

Fig. S3. ζ-potentials of the poly(MDPA-*co*-EDMA) monolithic column in dependence of buffer pH and relative charge of nature PA (D. Marsh, CRC handbook of lipid bilayers, CRC Press: Boca Raton, Florida, 1990.).

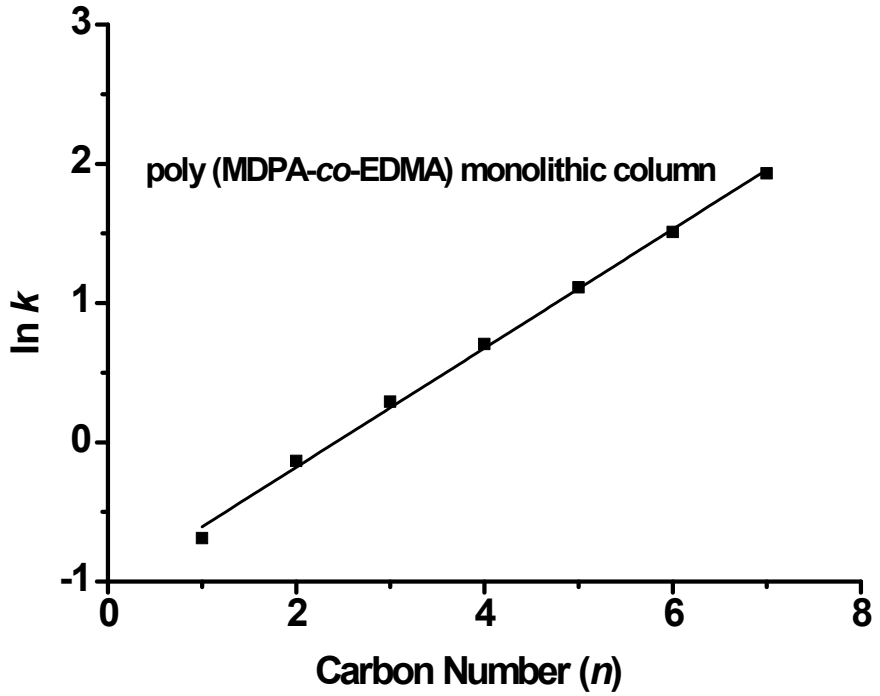

Fig. S4. Plots of logarithmic retention factor (In *k*) vs. the carbon number (*n*) of the alkyl substitutes of alkylphenones. Conditions: column dimensions, 140 mm × 100 μm I.D.; mobile phase, H_2O/ACN (50/50, v/v); flow rate, 600 nL/min; detection wavelength, 214 nm; samples, (1) acetophenone; (2) propiophenone; (3) butyrophenone; (4) valerophenone; (5) hexanophenone; (6) heptanophenone; (7) octanophenone.

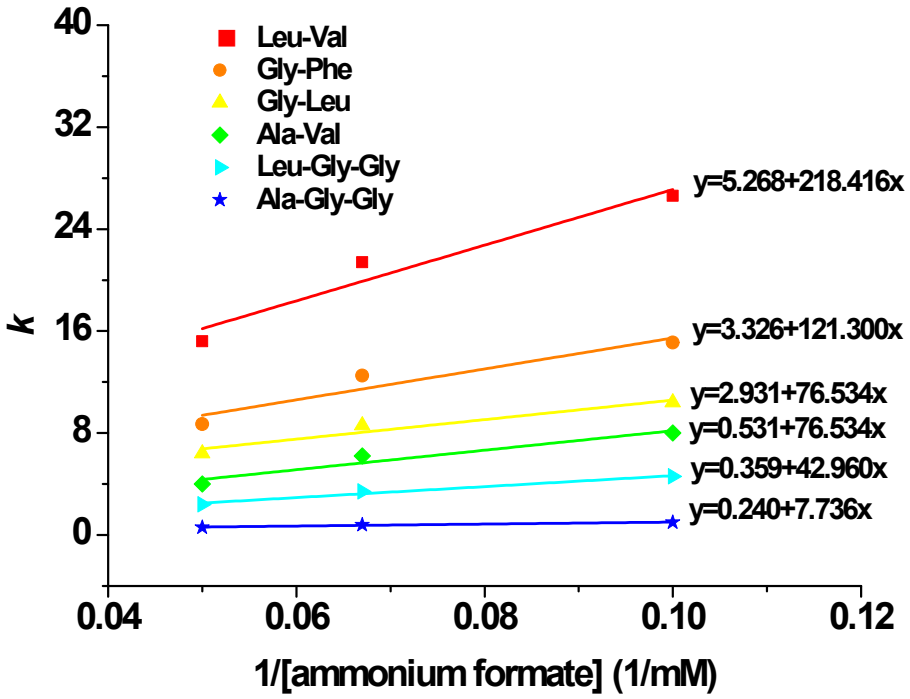

Fig. S5. The plot of *k* versus 1/[ammonium formate] for the separation of six small peptides on the poly(MDPA-*co*-EDMA) monolithic column. Condition: mobile phase, H_2O/ACN (90/10, v/v) containing ammonium formate pH 4.3 at various concentration; flow rate, 400 nL/min; detection wavelength, 214 nm; injection volume, 20 nL; Samples, (1) Ala-Gly-Gly; (2) Leu-Gly-Gly; (3) Ala-Val; (4) Gly-Leu; (5) Gly-Phe; (6) Leu-Val.

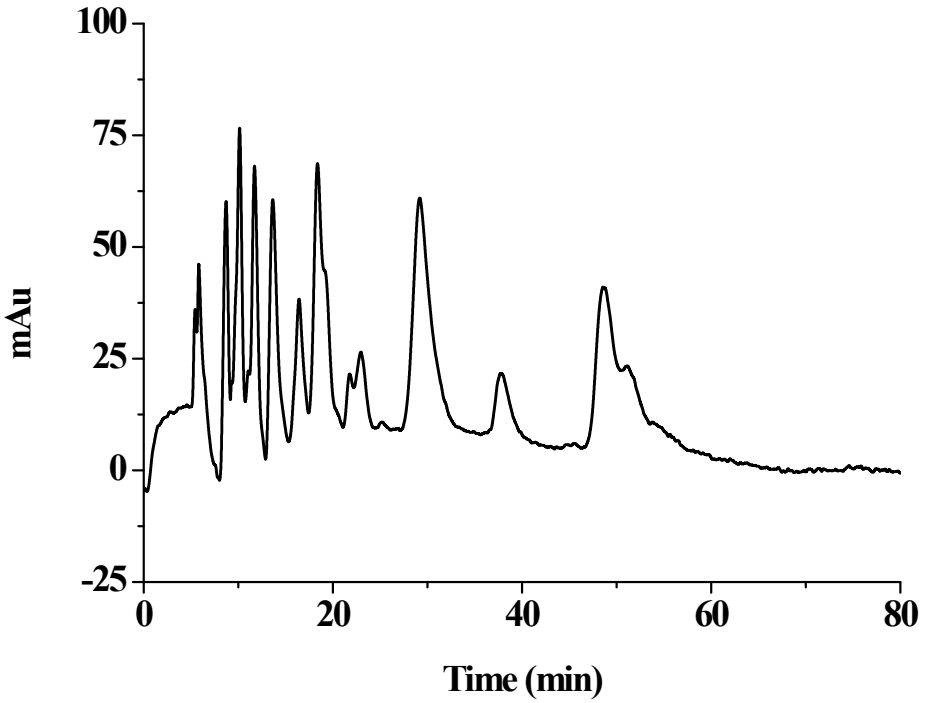

Fig. S6. Separation of Cyt C digest on the Poly(MDPA-*co*-EDMA) monolithic column. Conditions: column dimensions, 256 mm×100 μm I.D; Conditions: mobile phase: Eluent A: H_2O, 0.1% TFA; eluent B: ACN , 0.1% TFA; linear gradient, 0 min/30% B, 75.0 min/75% B, 76.0 min/30% B, 80 min/30% B; detection wavelength, 214 nm; flow rate: 600 nL/min; injection volume, 1 μL; Sample: Cyt C digest.

Table S1. Compositions of the polymerization mixtures used for the preparation of poly(MDPA-*co*-EDMA) monolithic columns and their properties.

Column	Monomers (%, w/w)		Porogens (%, w/w)		Monomers (%, w/w)	Porogens (%, w/w)	Column pressure (MPa)[a]	Column efficiencies (plates/m)[a]
	MDPA	EDMA	IPA	BDO				
C1	55	45	86	14	40	60	2.3	13000
C2	55	45	83	17	40	60	3.1	20000
C3	55	45	80	20	40	60	8.0	15000
C4	60	40	83	17	40	60	2.4	17000
C5	50	50	83	17	40	60	6.0	6700
C6	55	45	83	17	45	55	7.6	16000
C7	55	45	83	17	35	65	1.9	9200

Conditions: column dimension, 140 mm×100 μm I.D.; detection wavelength, 214 nm. [a] The column pressure was measured at linear velocity of 1 mm/s; mobile phase, H_2O/ACN (60/40, v/v); sample, anisole.

Table S2. Permeability of the poly(MDPA-*co*-EDMA) monolithic column.

Mobile phase	Relative polarity[a]	Viscosity η (10^{-3} Pa·s)[b]	Permeability *k* (10^{-13} m^2)
ACN	0.460	0.369	2.25
MeOH	0.762	0.544	1.54
50% H_2O/ACN	/	0.770	1.20
H_2O	1.000	0.890	0.09

[a] Relative polarity data were from http://virtual.yosemite.cc.ca.us/smurov/orgsoltab.htm.

[b] Viscosity data were from online CRC Handbook of Chemistry and Physics, 85th ed., CRC, Boca Raton, 2004-2005.

Table S3. Reproducibility of the poly(MDPA-*co*-EDMA) monolithic column.

	Retention factor, *k*			Peak area				Peak height			
	RSD_2 (%)	RSD_3 (%)	RSD_4 (%)	RSD_1 (%)	RSD_2 (%)	RSD_3 (%)	RSD_4 (%)	RSD_1 (%)	RSD_2 (%)	RSD_3 (%)	RSD_4 (%)
Run-to-run (n=10)	0.51	0.34	0.34	1.75	1.78	2.52	4.54	1.58	1.60	1.87	2.81
Day-to-day (n=3)	2.06	2.09	2.31	2.10	2.28	2.90	5.35	1.67	1.94	2.40	4.61
Column-to-column (n=3)	1.38	1.33	1.38	2.43	2.60	3.60	5.95	1.96	1.75	3.23	5.53
Batch-to-batch (n=3)	2.82	2.29	2.11	3.75	4.09	4.13	8.47	3.91	4.37	3.17	7.32

Conditions: column dimension, 140 mm×100 μm I.D.; mobile phase, H_2O/ACN (60/40, v/v); flow rate: 600 nL/min; detection wavelength, 214 nm; sample, 1. Thiourea, 2. Dimethyl phthalate, 3. Anisiole, 4. Naphthalene.

Table S4. Properties of small peptides (p*K*a and logD_{pH} values).

Analytes	pK_a[a]	log$D_{3.0}$[b]	log$D_{3.6}$[b]	log$D_{4.3}$[b]	log$D_{5.0}$[b]	log$D_{5.7}$[b]	log$D_{6.4}$[b]
Ala-Gly-Gly	3.59/8.21	-4.58	-4.39	-4.25	-4.19	-4.19	-4.20
Leu-Gly-Gly	3.55/7.66	-3.18	-2.98	-2.84	-2.79	-2.78	-2.78
Ala-Val	3.17/8.73	-3.03	-2.87	-2.80	-2.78	-2.78	-2.78
Gly-Leu	3.15/8.33	-2.84	-2.68	-2.61	-2.59	-2.59	-2.59
Gly-Phe	3.06/8.28	-2.38	-2.24	-2.18	-2.16	-2.16	-2.16
Leu-Val	3.17/8.65	-1.62	-1.46	-1.39	-1.37	-1.37	-1.37

[a] p*K*a values were calculated by the ExPASy Compute p*I*/Mw tool (http://web.expasy.org/compute_pi/).

[b] logD_{pH} values were calculated using Advanced Chemistry Development (ACD/Labs) Software version 11.02.

Table S5. Properties of pharmaceutical compounds (MW, p*K*a, log$D_{7.4}$ values).

Pharmaceutical compounds	MW (g/mol)	pK_a[a]	log$D_{7.4}$[b]
Aspirin	180.16	4.20	-1.69
Caffiene	194.19	10.4	-0.13
Sulfanilamide	172.20	10.6	-0.63
Benzenesulfonamide	157.19	10.1	/
Phenacetin	179.22	14.6	1.28
Coumarin	146.14	/	1.85
Ranitidine	314.40	8.20	-0.63
Sulpiride	341.43	8.90	-0.99

[a] p*K*a were calculated by the ExPASy Compute p*I*/Mw tool (http://web.expasy.org/compute_pi/).

[b] log$D_{7.4}$ values were calculated using Advanced Chemistry Development (ACD/Labs) Software version 11.02.