Supplementary material

A polyvinyl alcohol-coated silica gel stationary phase for hydrophilic interaction chromatography

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Experimental section

Reagents and materials

Spherical silica was purchased from Fuji (Fuji Silysia Chemical Ltd., Japan, 5 μm particle size; 300 m^2 /g). PVA (MW 1750±50) was from Lingfeng Corp. (Shanghai, China). Model analytes mentioned below are from Aladdin Ltd. (Shanghai, China). For comparison, two commercial HILIC columns were from Acchrom (Beijing, China), including XAmide and Diol with size of 150 mm length× 4.6 mm i.d. ×5 μm dia. Unless otherwise stated, methanol was used for sample preparation.

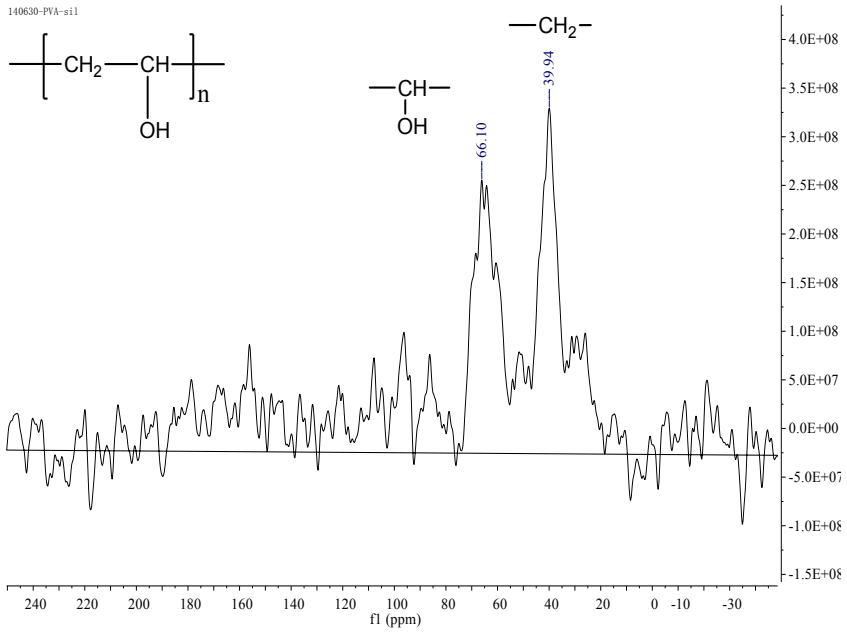
Instrumentation

The chromatographic separation was performed on a Waters Alliance HPLC consisting of a 2695 separation unit, automatic injector and a 2998-UV absorbance detector. Unless otherwise stated, the flow rate was1 mL/min and the detection wavelength was 254 nm.

The morphology of stationary phase was characterized by a scanning electron microscope (FESEM NOVA NanoSEM 450, FEI, USA). The pore structure and surface area were measured by a TriStar II 3020TM Surface Area and Pore Analyzer (Micromeritic, USA). The FT-IR spectrum was recorded on a spectrum 100 (PerkinElmer, USA). The chemical structure of stationary phase was characterized by an AVANCE 50013C-CP/MAS NMR spectroscopy (Bruker, Germany). Subcritical fluid chromatography was performed on Waters AcQuity UPC2 TM (Waters Corp., USA).

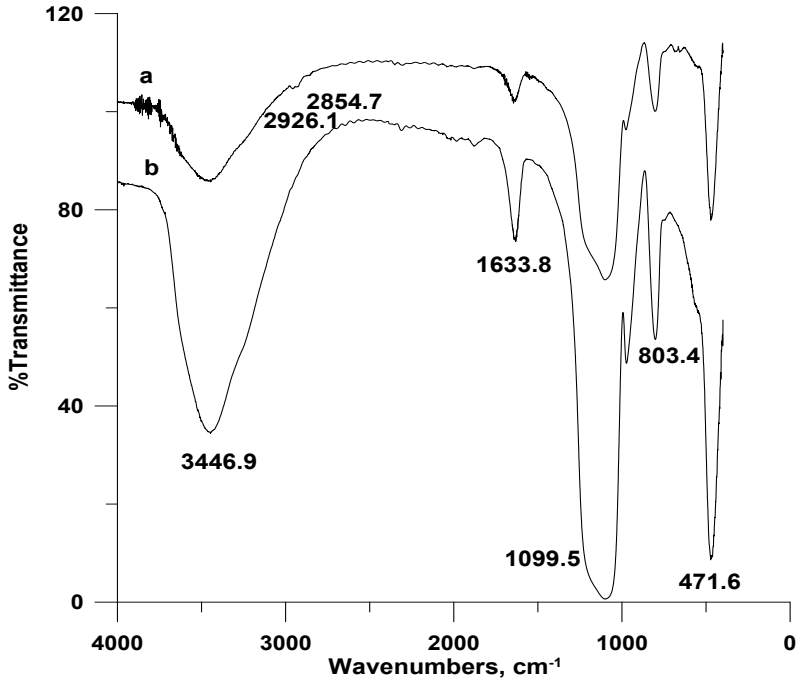
SI-Table 1.Characterization of stationary phases.

Material	BET surface area (m^2/g)	C%	Plate count
Bare silica	318.35	nd	/
PVA-Sil- F	277.40	2.74	39378
PVA-Sil-T(1 layer)	276.68	3.04	74715
PVA-Sil-T (2 layer)	/	4.65	67438
PVA-Sil-T(3 layer)	/	7.60	61199



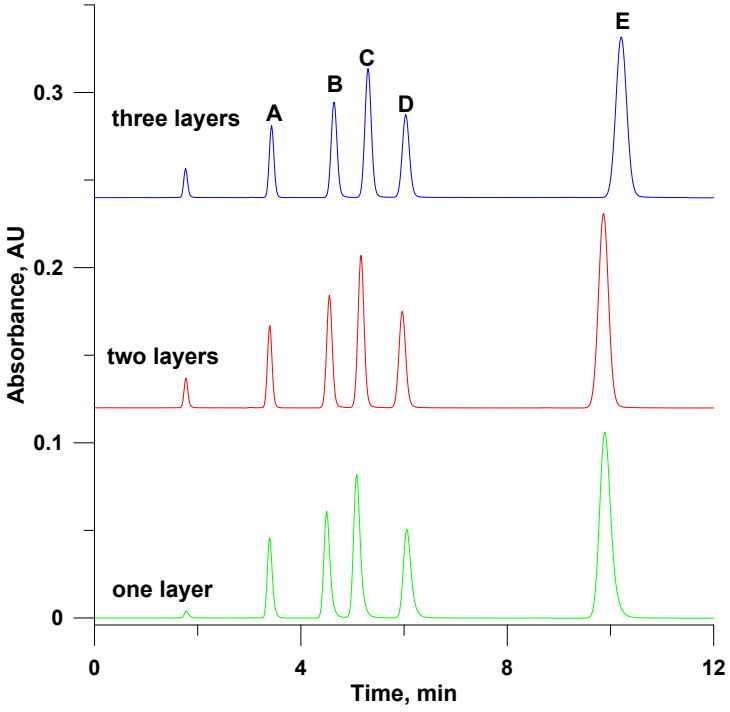
SI-Fig. 1 Solid phase ^{13}C-CP/MAS NMR spectrogram of PVA-Sil-T

The peaks at δ= +66.01ppm and δ= +39.94ppm were attributed to -CH-OH and -CH_2-, respectively. The peak assignment referred to previous report.[1-2]



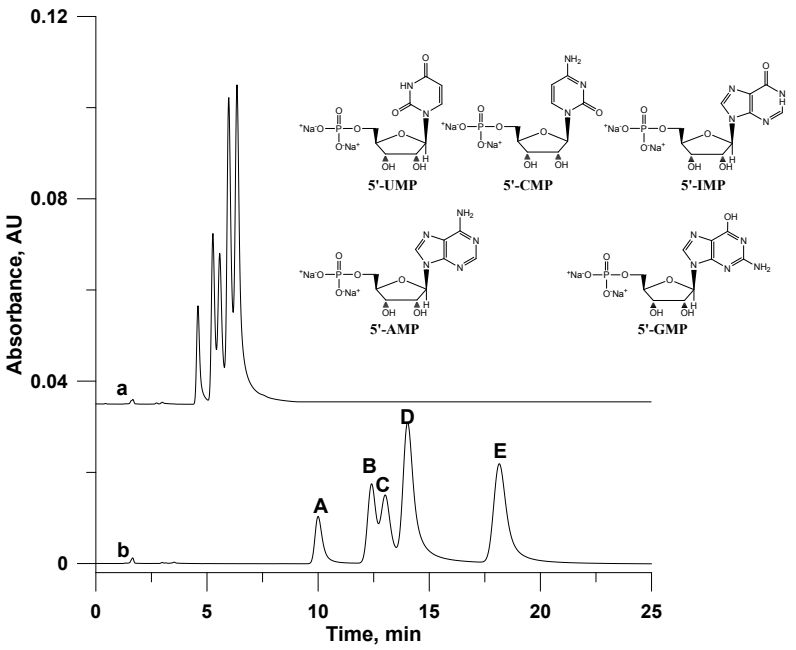
SI-Fig.2 IR spectra of PVA-Sil-T (a) and bare silica gel (b).

The characteristic peaks of bare silica (e.g. 3446.9 cm^{-1}, 1633.8 cm^{-1}, 1099.5 cm^{-1}) were obviously weakened relative to PVA-Sil-T, which resulted from the existence of PVA coating.



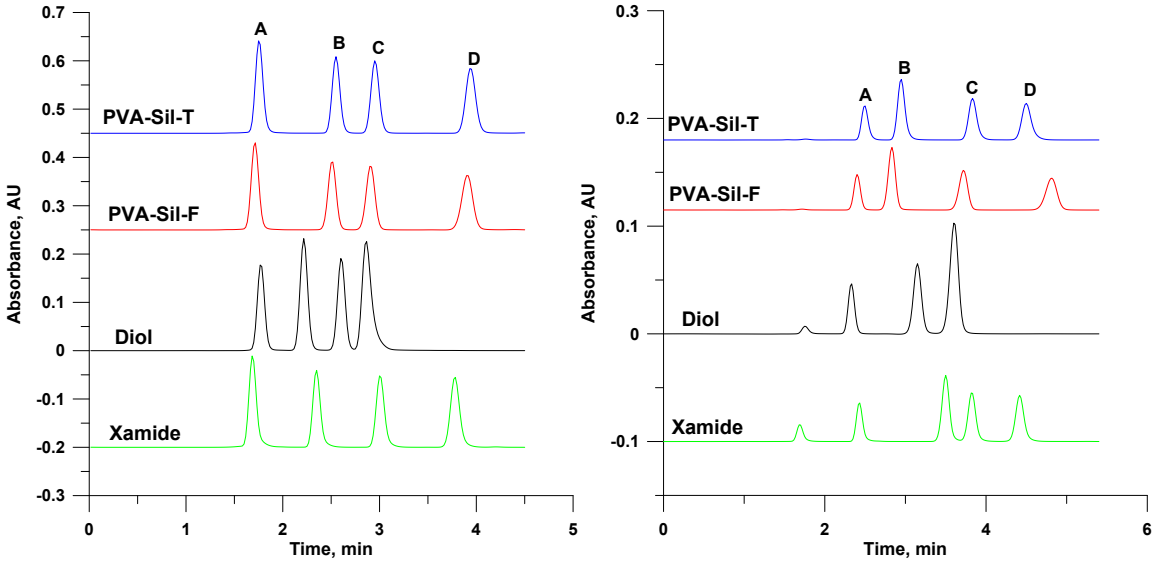
SI-Fig.3 Typical chromatogram by PVA-Sil-T with different layers of PVA coating.

Conditions: mobile phase: A, ACN; B, H_2O; C, 250 mM NH_4FA (pH 3.5). 90% A/4% B/6% C; injection volume, 10 µL; column temperature, 30°C. Peak identification: A, uracil, B, 5-methyl uridine, C, uridine, D, adenine, E, cytosine. Peak order is same for all tested columns.



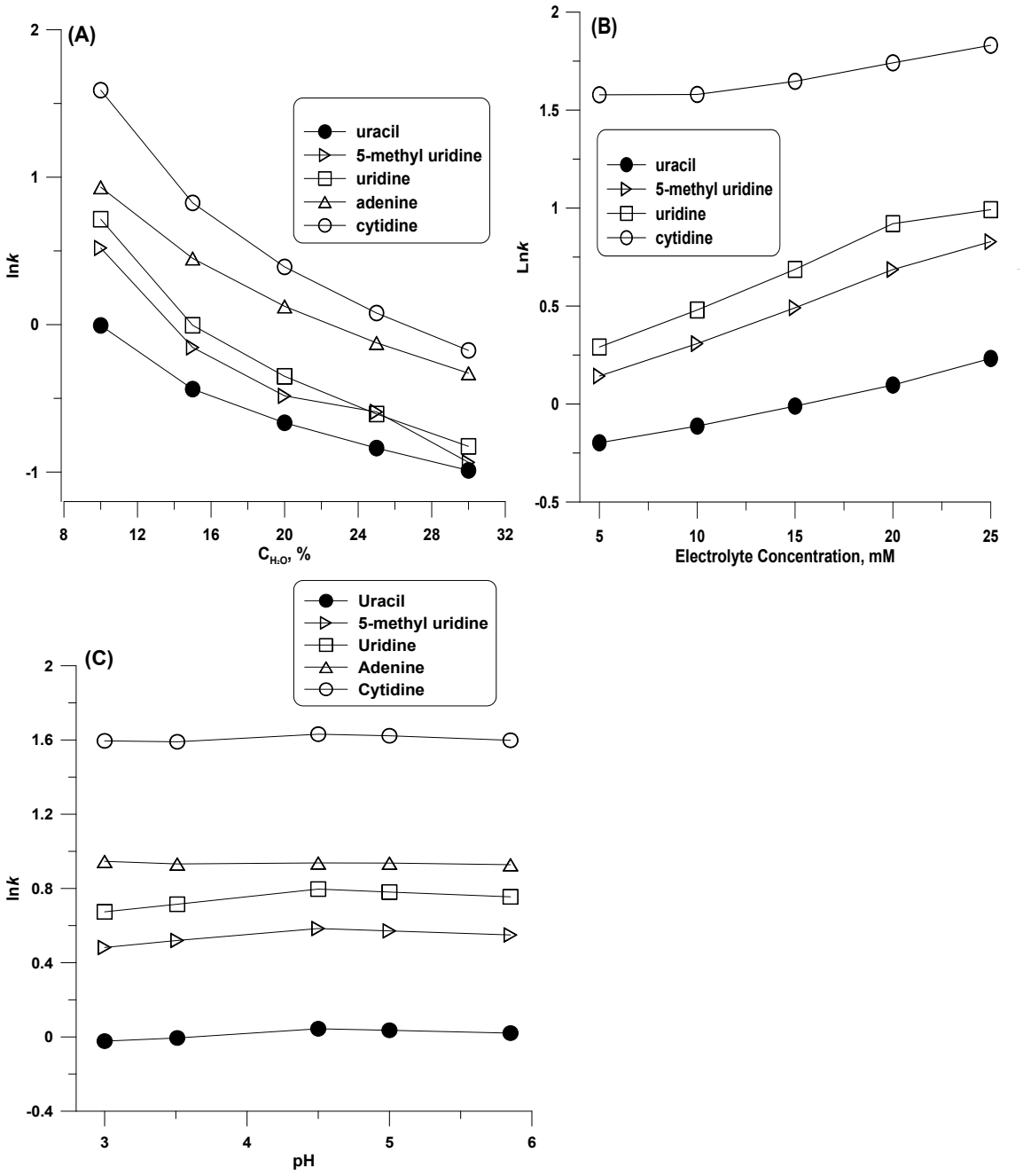
SI-Fig.4 Separation of nucleotides on Diol (a) and PVA-Sil-T (b).

Conditions: mobile phase: acetonitrile/100 mM CH_3COONH_4 (75/25); flow rate, 0.2 mL/min; temperature, 40°C. Peak identification: A,5'-UMP, B, 5'-IMP, C, 5'-AMP, D,5'-GMP, E, 5'-CMP.Peak order is same for a and b columns.



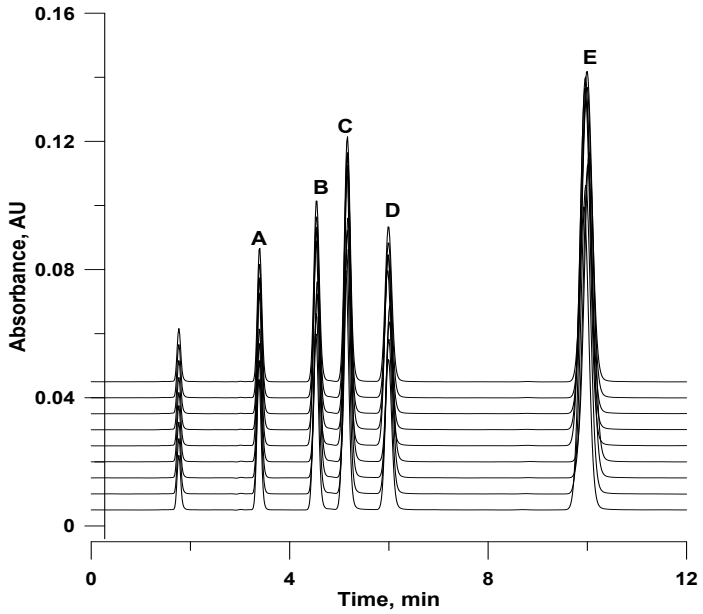
SI-Fig.5 Typical chromatogram of organic bases (left) and organic acids(right).

Other conditions same to Fig. 3. Peak identification, organic bases (left), A: diphenylamine, B: caffeine, C: theophylline, D: diprophylline; (right), A: salicylic acid, B: sorbic acid, C: acetyl salicylic acid, D: phthalic acid. Peak order is same for all tested columns.



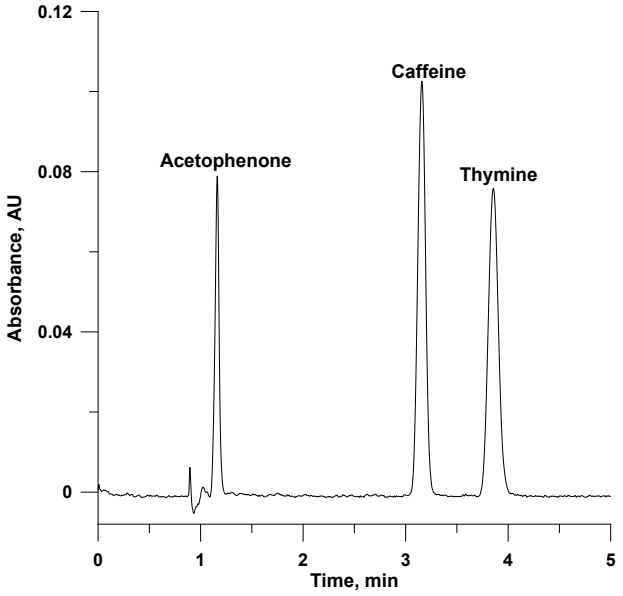
SI-Fig.6 Exploration of HILIC retention mechanism.

Conditions: (A), mobile phase, A: ACN; B: H_2O; C: 250 mM NH_4FA (pH=3.5) with water concentration range of10% to 30% (v/v); other conditions same to Fig. 3; (B): A,ACN; B, H_2O; C: 250 mM NH_4FA (pH=3.51) with concentration of 5-25 mM; other conditions same to Fig. 3; (C): A, ACN; B, H_2O; C: 250 mM NH_4FA (pH value varying from 3.1 to 5.7); other conditions same to Fig. 3.



SI-Fig.7 Reproducibility demonstration of nucleosides on PVA-Sil-T.

Peak identification: A: uracil, B: 5-methyl uridine, C: uridine, D: adenine, E: cytosine. Other conditions same to Fig.3.



SI-Fig.8 Typical chromatogram of PVA-Sil-T in SubFC.

Conditions: mobile phase: A: CO_2; B: methanol. 80% A/20% B; detection wavelength, 230 nm; flow rate, 2.0 mL/min; injection volume, 5 μL; column temperature; 40°C; backpressure, 2000 psi.

References

[1] Y. Lu, Q.M. Kong, R. Jing, X. Hu, et al. Polym. Degrad. Stab.,2013, 98, 1103.

[2] J. A. Killion, L. M. Geever, M. Cloonan, et al. J. Polymer Research, 2014, 21,1.