

## Supporting information

for

### **One-step synthesis of organic-inorganic hybrid boronate affinity monolithic column with synergistic co-monomers**

**Qin Yang,<sup>a,b</sup> Dihui Huang,<sup>a</sup> Shanxia Jin,<sup>a</sup> Hailong Zhou,<sup>a</sup> Ping Zhou<sup>\*a</sup>**

<sup>a</sup> *Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education),  
College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China*

<sup>b</sup> *College of Pharmacy, Hubei University of Chinese Medicine, Wuhan, P. R. China*

\* To whom correspondence should be addressed: Dr. Ping Zhou, phone/fax 86-27-68752136,  
*E-mail:* zbping@whu.edu.cn.

## Experimental sections

### 1. Materials and Instrumentation

#### 1.1 Materials

4-Vinylphenylboronic acid (VPBA), adenosine, and 2-deoxyadenosine were purchased from Sigma (St. Louis, MO, USA). Tetraethoxysilane (TEOS),  $\gamma$ -methacryloxypropyltrimethoxysilane ( $\gamma$ -MAPS) and *N*-( $\beta$ -aminoethyl)- $\gamma$ -aminopropyltriethoxysilane (AEAPTES) were products of Wuhan University Silicone New Material (Wuhan, China). 2, 2-Azobisisobutyronitrile (AIBN) was obtained from Tianjin Chemistry Reagent Factory (Tianjin, China) and recrystallized in methanol prior to use. Cetyltrimethylammonium bromide (CTMAB), sodiumdihydrogenphosphate ( $\text{NaH}_2\text{PO}_4$ ), disodium hydrogenphosphate ( $\text{Na}_2\text{HPO}_4$ ), potassium dihydrogenphosphate ( $\text{KH}_2\text{PO}_4$ ), quinol, catechol, resorcinol, HPLC grade methanol (MeOH) were obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were of analytical grade or better. Fused-silica capillaries (250  $\mu\text{m}$  i.d. and 375  $\mu\text{m}$  o.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

#### 1.2 Instrumentation

$^{11}\text{B}$  NMR spectra were recorded on a Mercury vx-300 instrument (Varian, Salt Lake City, UT, USA) operated at 96.277 MHz and 25°C. 4-VPBA (10.0 mg) was dissolved in  $\text{CDCl}_3$  (0.2 mL) with or without the addition of AEAPTES (0.02 mL) for measurement. An organic-inorganic hybrid affinity monolithic column with a total length of 30 cm was used unless otherwise stated. All chromatographic experiments were performed on a LC-20AD miniaturized Liquid Chromatography (Shimadzu, Kyoto, Japan) with a UV Detector 2500 (Knauer, Berlin, Germany). A flow rate of 0.01 mL/min was used unless otherwise stated and the UV absorbance was monitored at 275 nm or 254 nm. The signal was fed into an EC2000 chromatography workstation (Dalian Elite Analytical Instruments, Dalian, China). Fourier transform infrared (FT-IR) spectra were acquired on a Thermo Scientific Nicolet iS10 FT-IR spectrometer (Waltham, MA, USA). Samples were injected through an injection valve with a 0.2  $\mu\text{L}$  sample loop which was made of a total length of 10 cm fused-silica capillaries (50  $\mu\text{m}$  i.d. and 375  $\mu\text{m}$  o.d.). Morphology of the monolithic column was examined by Quanta 200 scanning electron microscopy system (Philips-FEI, Eindhoven, Netherlands). The surface area and mesopore size distribution were measured by nitrogen adsorption/desorption experiments with an ASAP-2020 Plus surface area and pore size analyzer (Micromeritics, Norcross, GA, USA). Prior to measurement, the VPBA-silica monolith was synthesized in a tube of 0.5 cm i.d. with the same procedure as that for the preparation of the capillary monolith. Then the monolithic materials were cut to 0.5-cm-long pieces, and the physically adsorbed substances, EtOH and porogens were extracted by MeOH using Soxhlet extraction. The specific surface area values were calculated according to the Brunauer-Emmett-Teller (BET) equation. The pore size was evaluated from the desorption branches of isotherms based on Barrett-Joyner-Halenda (BJH) model. X-ray diffraction (XRD) pattern was recorded on an X-ray diffractometer (XRD, PANalytical, Netherlands) with  $\text{CuK}\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) operated at 40 kV and 50 mA. The data were collected in a step of  $0.026^\circ \text{ s}^{-1}$  with the scattering angles ( $2\theta$ ) ranging from  $10^\circ$  to  $80^\circ$ .

## 2. Preparation of the B-N silica hybrid affinity monolithic column

The capillary (250  $\mu\text{m}$  i.d. and 370  $\mu\text{m}$  o.d.) was rinsed with 0.1 M NaOH for 2 h, 0.1 M HCl for 2 h, water for 1h, and ethanol for 1h, and then dried under a stream of nitrogen for 3 h. The pretreated capillary was stored at 40  $^{\circ}\text{C}$  prior to use. The monolithic column was prepared as follows: 225  $\mu\text{L}$  ethanol, 75  $\mu\text{L}$  water, 0.0111 g CTMAB and 0.0060g 4-VPBA were mixed in 1.5 mL eppendorf tube and ultrasonicated for 5 min at 0 $^{\circ}\text{C}$ . A volume of 160  $\mu\text{L}$  TEOS, 20  $\mu\text{L}$  AEAPTES and 20  $\mu\text{L}$   $\gamma$ -MAPS were then added to the above mixture and ultrasonicated for 30 s at 0  $^{\circ}\text{C}$ . Finally, the homogenous solution was filled into the pretreated capillary to an appropriate length with a syringe. When both ends of the capillary were sealed with two pieces of rubbers, the capillary was incubated at 60 $^{\circ}\text{C}$  for 12 h. The obtained monolithic column was flushed with MeOH and water to remove the residual monomers and porogens.

In our previous study<sup>1</sup>, we prepared a hybrid silica column by TEOS and AEAPTES. A ratio of siloxane (TEOS)/organosiloxane (AEAPTES) 4:1 v/v was found to give the best performance. In present approach, three precursors TEOS, AEAPTES and  $\gamma$ -MAPS were used. Because of the differences in rates of hydrolysis and condensation among different precursors, the synthetic conditions for hybrid silica monolithic structures had to be controlled very carefully<sup>2</sup>. While siloxane and organosiloxane was kept a constant ratio of 4:1 v/v, various ratios of AEAPTES/ $\gamma$ -MAPS were chosen to prepare the monolith. Homogenous monolith with good permeability was obtained when the ratio of AEAPTES/ $\gamma$ -MAPS was 1:1 v/v. The  $\gamma$ -MAPS played a key role in incorporating the boron functional moiety, but the monolith becomes more brittle with an increase in  $\gamma$ -MAPS. Larger amount of 4-VPBA was expected to give higher column capacity for target analytes. However, as shown in table S1, too much 4-VPBA added would impact the state of the monolith even the formation of the skeleton.

## 3. Permeability ( $K_{p,F}$ ) of the B-N silica hybrid affinity monolithic column

A linear relationship between pressure and mobile phase velocity was always observed. Permeability ( $K_{p,F}$ ) was further determined by liquid chromatography in 100% water. Calculations were made according to Darcy's equation for porous beds<sup>3,4</sup> in relation to the superficial velocity:

$$K_{p,F} = (F/S)(\eta L)/\Delta P \quad (1)$$

where,  $F$  is the mobile phase flow rate,  $S$  is the internal cross section of the capillary column,  $\eta$  is the viscosity of the mobile phase,  $L$  is the column length and  $\Delta P$  is the column pressure drop. Mobile phase  $\text{H}_2\text{O}$ ;  $\eta_{\text{H}_2\text{O}} = 1.01 \times 10^{-3} \text{ Pa}\cdot\text{s}$  (20 $^{\circ}\text{C}$ ); flow rate: 2  $\mu\text{L min}^{-1}$ ;  $L = 20 \text{ cm}$ ;  $S = \pi r^2$  ( $r = 125 \mu\text{m}$ ).  $\Delta P = 33 \text{ psi}$ . The permeability ( $K_{p,F}$ ) of the monolithic column was about  $33.2 \times 10^{-14} \text{ m}^2$ .

## 4. Dynamic binding capacity measurement

To determine and compare the dynamic binding capacity of the VPBA-silica hybrid affinity monolith for non-*cis*-diol biomolecules and *cis*-diol biomolecules in neutral media, frontal analysis of the monolith was carried out with 0.1  $\text{mg mL}^{-1}$  quinol and 0.2  $\text{mg mL}^{-1}$  catechol dissolved in 10 mM phosphate buffer (pH 7.0), respectively. The binding capacity ( $Q$ ) was calculated by the equation (2).<sup>5</sup>

$$Q = (t_R - t_0) F \times C / V \quad (2)$$

Under neutral media, while quinol was not captured by the monolith and thus eluted first ( $t_0$ ) as the dead time marker, catechol was captured by the monolith and eluted later ( $t_R$ ) until the

monolith was saturated.  $C$  is the catechol concentration ( $0.2 \text{ mg mL}^{-1}$ ) and  $V$  is the volume of monolithic column.  $F$  is flow rate of elution buffer. In brief, the VPBA-silica hybrid affinity monolith was equilibrated with a loading buffer (elution buffer). The sample solution containing  $0.1 \text{ mg mL}^{-1}$  quinol or  $0.2 \text{ mg mL}^{-1}$  catechol was pumped through the column. After equilibration, elution of catechol and regeneration of the monolith were carried out with the elution buffer. The dynamic binding capacity of the silica hybrid affinity monolith for catechol was  $10.61 \text{ } \mu\text{mol L}^{-1}$ .

### 5 Analysis of urinary sample on the B-N silica hybrid affinity monolithic column

The urine was spontaneously collected from a healthy male adult. The human urinary sample was frozen immediately and stored at  $-20^\circ\text{C}$ . Prior to analysis, the sample was thawed at room temperature. A 10 mL aliquot of urine was centrifuged for 15 min at 15,000 rpm. The supernatant was collected and the pH value was adjusted to 7.0 by ammonia (28%).<sup>6</sup> The pretreated urine was separated by the hybrid affinity monolithic capillary. The mobile phase was 10 mM phosphate buffer (pH 7.0), and the flow rate was  $8 \text{ } \mu\text{L min}^{-1}$ . The detection wavelength was set at 254 nm. After 20 min, the mobile phase was changed to 100 mM HAc (pH 2.7). Then, the eluate corresponding to the affinity captured fraction was collected and lyophilized for further liquid chromatographic analyses.

### 6 Chromatographic separation of the extracted fraction by RP-HPLC

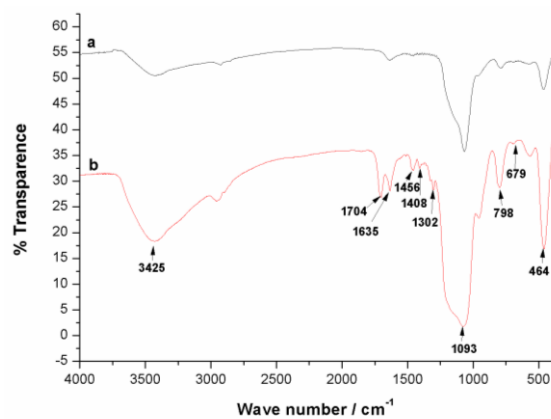
The analyses were carried out by RP-HPLC on an Agilent 1100 Series LC system (Agilent Technologies, CA, USA) with Agilent HC-C18 column ( $4.6 \times 250 \text{ mm}$ ). The mobile phase was 25 mM potassium dihydrogenphosphate (pH 4.5) and MeOH. From 0 to 6 min, there was 10% MeOH, and linear gradient from 10% to 50% MeOH was carried out in 9 min, and then MeOH was increased from 50% to 100% in 5 min, and held for 15 min. The flow rate was  $1.0 \text{ mL min}^{-1}$  and the detection wavelength was set at 254 nm. Prior to analysis, the lyophilized extract was dissolved in mobile phase (90:10 v/v). The diluted urinary samples with and without extraction by the boronate affinity column were analyzed by RP-HPLC. The chromatograms are shown in Fig. S5, and the results reveal that some components such as peak 3 and 4 were apparently enriched by the boronate affinity column. Identity for some peaks was confirmed by comparison the chromatogram with that for known standards.

## Supporting Table and Figures

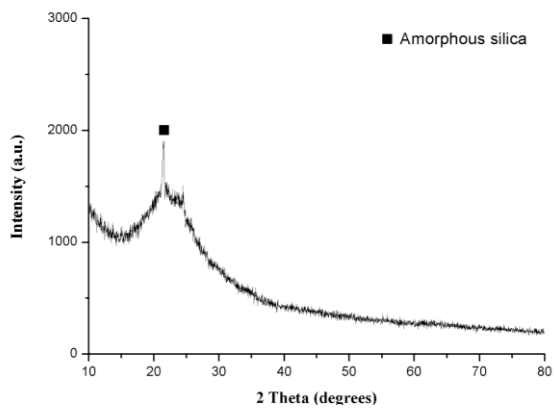
**Table S1** Effect of synthesis parameters on the formation of VPBA-silica hybrid affinity monoliths

	4-VPBA (mg)	CTAMB (mg)	State of column	Back pressure (psi)	Permeability $K$ ( $\times 10^{-14} \text{ m}^2$ )
M 1	4.0	8.8	Homogeneous	Block	
M 2	4.0	11.1	Slack		
M 3	4.0	22.2	Slightly shrunked		
M 4	6.0	8.8	Homogeneous	700	2.8
<b>M 5</b>	<b>6.0</b>	<b>11.1</b>	<b>Homogeneous</b>	<b>60</b>	<b>33.2</b>
M 6	6.0	16.7	Slack, slightly detached		
M 7	6.0	22.2	Sol state		
M 8	8.0	11.1	Slack		
M 9	8.0	22.2	Slack		
M 10	10.0	11.1	Sol state		
M 11	12.0	11.1	Sol state		

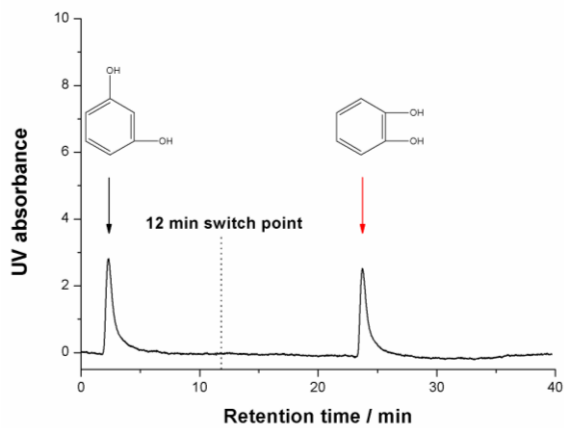
Flushed with  $\text{H}_2\text{O}$  ( $\eta_{\text{H}_2\text{O}} = 1.01 \times 10^{-3} \text{ pa}\cdot\text{s}$ ,  $20^\circ\text{C}$ ); flow rate,  $2 \mu\text{L min}^{-1}$ ;  $L=20 \text{ cm}$ ;  $S = \pi r^2$  ( $r=125 \mu\text{m}$ )



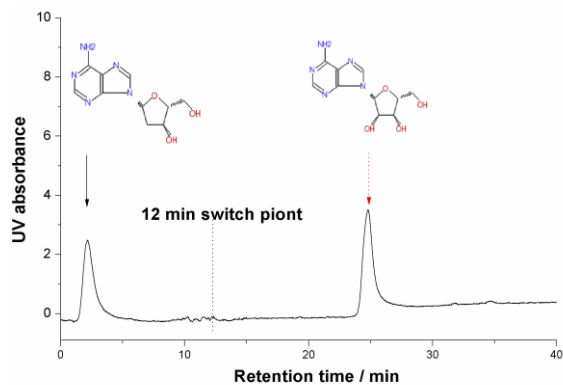
**Fig. S1** FT-IR spectra of (a) the amino-silica-based monolithic skeleton after polymerization reaction and (b) the amino-VPBA-silica hybrid affinity monolith after polymerization reaction.



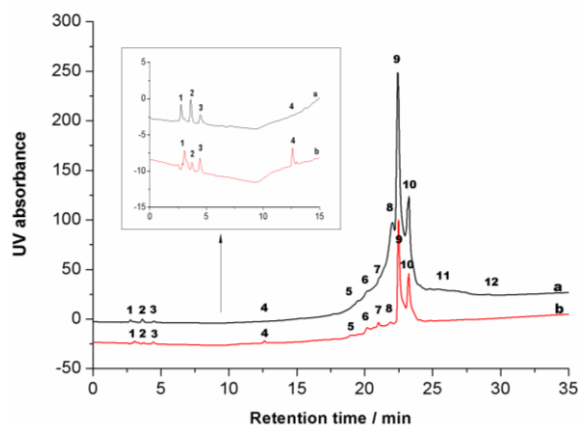
**Fig. S2.** The wide-angle XRD pattern of the amino-VPBA-silica hybrid affinity monolith.



**Fig. S3** Chromatographic retention of resorcinol and catechol on the boronate-functionalized monolithic column. Mobile phase: 10 mM sodium phosphate buffer (pH 7.0), the mobile phase was changed to 100 mM HAC (pH 2.7) at 12 min. Flow rate: 0.01 mL min<sup>-1</sup>; Detection wavelength: 275 nm. Sample: resorcinol and catechol were dissolved in the 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.1 mg mL<sup>-1</sup> each.



**Fig. S4** Chromatographic retention of adenosine and desoxyadenosine on the boronate-functionalized monolithic column. Mobile phase: 10 mM sodium phosphate buffer (pH 7.0), the mobile phase was changed to 100 mM HAc (pH 2.7) at 12 min. Flow rate: 0.01 mL min<sup>-1</sup>; Detection wavelength: 254 nm. Sample: adenosine and desoxyadenosine were dissolved in the 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 0.1 mg mL<sup>-1</sup> each.



**Fig. S5** RP-HPLC analysis of diluted urine sample (a) and extracted components by hybrid boronate affinity monolithic column (b). Mobile phase: A was 25 mM potassium dihydrogenphosphate (pH 4.5) and B was MeOH; from 0 to 6 min, 10% (v/v) B, then linear gradient from 10% to 50% B in 9 min, and linear gradient from 50% to 100% B in 5 min, and then held for 15 min; Flow rate: 1.0 mL min<sup>-1</sup>; Detection wavelength: 254 nm. Peak identity: 3, uridine; 4, adenosine; other, unknown.

## References

1. S. Yu, J. Geng, P. Zhou, J. Wang, A. R. Feng, X. Chen, H. Tong and J. M. Hu, *Anal. Chim. Acta*, 2008, **611**, 173–181.
2. L. L. Hench and J. K. West, *Chem. Rev.*, 1990, **90**, 33-72.
3. C. A. Cramers, J. A. Rijks and C. P. M. Schutjes, *Chromatographia*, 1981, **14**, 439–444.
4. M. A. Jaoude and J. Randon, *Anal. Bioanal. Chem.* 2011, **400**, 1241-1249.
5. M. Y. Wang, J. Xu, X. Zhou and T. W. Tan, *J. Chromatogr. A*, 2007, **1147**, 24-29.
6. H. Li, H. Wang, Y. Liu and Z. Liu, *Chem. Commun.*, 2012, **48**, 4115-4117.