



# Preparation and chromatographic evaluation of hydrophilic polymer brushes grafted-silica with post modification of silicon/carbon dots as a green liquid chromatography stationary phase

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Received: 5 June 2024 / Accepted: 17 July 2024 / Published online: 30 July 2024  
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## Abstract

Polar stationary phases were prepared by grafting hydrophilic acrylamide (Am) polymer brushes with post modification of carbon dots (CDs) and silicon dots (SiDs) onto SiO<sub>2</sub> particles. The prepared stationary phases, SiO<sub>2</sub>-PAm-CDs, SiO<sub>2</sub>-PAm-CDs/SiDs, and SiO<sub>2</sub>-PAm-SiDs, were packed as chromatographic columns, respectively. Using nucleic bases, organic acids, and  $\beta$ -agonists as target substances to investigate the influence of chromatographic conditions on retention and separation, the packed columns showed the partitioning and adsorption of mixed retention behavior in hydrophilic interaction liquid chromatography mode and successfully separated the polar compounds. Most importantly, under *per* aqueous liquid chromatography mode (using 100% water as mobile phase), those columns still had good separation ability toward nucleic bases,  $\beta$ -agonist, and organic acids. Because AM is a temperature-sensitive monomer, the resulting van't Hoff curves exhibited a nonlinear relationship, having temperature-responsive chromatographic characteristic under pure water separation. Hence, building on temperature-sensitive characteristics and pure water of separation conditions, the separation selectivity toward hydrophilic compounds greatly improved. Compared with the commercial hydrophilic columns, the efficiency of our developed column had the superior ability in separation and detection of betaine in Goji berry with the enhanced resolution achieved in the proposed green separation method (just using pure water as mobile phase).

**Keywords** *Per* aqueous liquid chromatography · Green separation · Temperature-responsive · Goji berry

## Introduction

Nowadays, the frontier fields, such as pharmacology, environmental science, and materials science, continue to evolve, so the research focus of analytical chemistry shifts to analyze complex sample [1]. Hydrophilic interaction chromatography (HILIC) has received a significant interest with great success in the separation of biologically active compounds, such as pharmaceuticals, nucleoside, amino acids, peptides, proteins, neurotransmitter, oligonucleotides, and carbohydrates [2]. But in HILIC, the separation is always conducted on over 80–95% percent of acetonitrile (ACN, organic solvent-rich mobile phase), resulting in a large amount of toxic and hazardous organic waste to influence human health and the environment [3]. With the concept of green chemistry becoming more and more popular, *per* aqueous liquid chromatography (PALC), as a sub-technique of HILIC, is considered a green LC because of using water-rich eluents (over 90% aqueous solution). To date, PALC has attracted attention for its great value in ecology and economy [4].

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Currently, the development of a chromatographic stationary phase, which possesses excellent separation efficiency using an environmentally friendly mobile phase, is necessary and challenging work. However, literature reported that HILIC and PALC stationary phases are very limited in the past decades, especially for the PALC stationary phase. Normally, the stationary phases of HILIC and PALC are polar stationary phases, and their synthesis is always adopted by modifying polar or hydrophilic ligands on the surface of the substrate. Hydrophilic ligands can be roughly classified into three kinds, such as polar molecules (i.e., undecylenic acid, maltose, and zwitterionic compound) [5, 6]; hydrophilic polymers (i.e., polysaccharide, polyethyleneimin, polyethylene glycol, and polymeric ionic liquids) [7]; and nanomaterials (i.e., Au and carbon nanoparticles) [8]. Beyond that, in synthesis, the modification of more hydrophilic functional groups on the surface of the stationary phase could enable better formation of a water-rich layer, resulting in better separation selectivity to polar compounds [9]. So, for the separation of polar compounds with good selectivity, it is an efficient method to develop novel polar stationary phase by exploring novel hydrophilic ligand and improving their bonding amounts.

Novel hydrophilic species, carbon quantum dots (CDs) and silica quantum dots (SiDs), have the superior properties of good biological compatibility, low toxicity, and high stability to focus much attention in recent years [10, 11]. Here, in view of the developed polar stationary phase, CDs and SiDs could be intrigued as promising hydrophilic ligands of their unique properties [12, 13]: (i) preparation is easy from molecular precursors; (ii) large quantities of polar groups, such as amine, hydroxy, and carboxyl groups, exists on their surfaces; after modifying into stationary phase, they can endow abundant hydrophilic interaction sites to polar compounds; (iii) small particle and regularly sphere-like morphology could make no affection of uniformity of chromatographic packing, avoiding high column pressure and poor separation; (iv) different from the routine carbon nanomaterials, CDs can avoid aggregation to easily incorporate hydrophilic surface, while SiDs have favorable compatibility with SiO<sub>2</sub> sphere (the classical substrate of stationary phase) to easily introduce Si–OH, as such, the introduction of CDs and SiDs into stationary phase could unquestionable strength its hydrophilic nature. Conclusively, CDs and SiDs are considered promising hydrophilic ligands for the polar stationary phase, and in recent years, CDs have been utilized to exploit the new stationary phase; the existing researches have manifested their excellent efficiency in separation [14, 15]. However, objectively speaking, SiDs as hydrophilic species for devised stationary phase have few reported till date.

Besides, in theory, the higher density of surface hydrophilic groups could form the thicker water-rich layer,

having better hydrophilic selectivity [16]. Therefore, for boosting separation efficiency of polar stationary phase, it is an effective strategy to combine the high-density hydrophilic polymer with high-affinity hydrophilic ligands. In our current works [17, 18], it has been confirmed that the novel bonding technique, atom transfer radical polymerization (ATRP), is an efficient way to control the density of functional ligand in preparation for the stationary phase. Here, the hydrophilic monomer, acrylamide (Am), was first grafted on the SiO<sub>2</sub> matrix by ATRP. CDs (glucose and  $\beta$ -alanine as precursors) and SiDs (sodium citrate and 3-(2-aminoethylamino) propyldimethoxymethylsilane as precursors) were rapidly prepared by hydrothermal method, then CDs, CDs/SiDs, and SiDs were bonded at poly (Am) (PAm) brushes terminal by nucleophilic substitution, obtaining three new stationary phases, named as SiO<sub>2</sub>-PAm-CDs, SiO<sub>2</sub>-PAm-CDs/SiDs, and SiO<sub>2</sub>-PAm-SiDs, respectively (Fig. 1). These stationary phases were suitable for separating nucleic bases, organic acids, and  $\beta$ -agonists under HILLIC mode (ACN-rich mobile phase) and PALC mode (pure water separation) but exhibited the differentiated separation selectivity to those polar compounds. Indeed, the hydrophilic PAm combined with the high affinity of CDs or SiDs ligands has a cooperation effect to boost the chromatographic performance of polar stationary phases, showing the superior potential in detection of betaine from goji berry using pure water of green separation condition.

## Experimental

### Materials and reagents

All the materials and reagents are detailed in the Supplementary information.

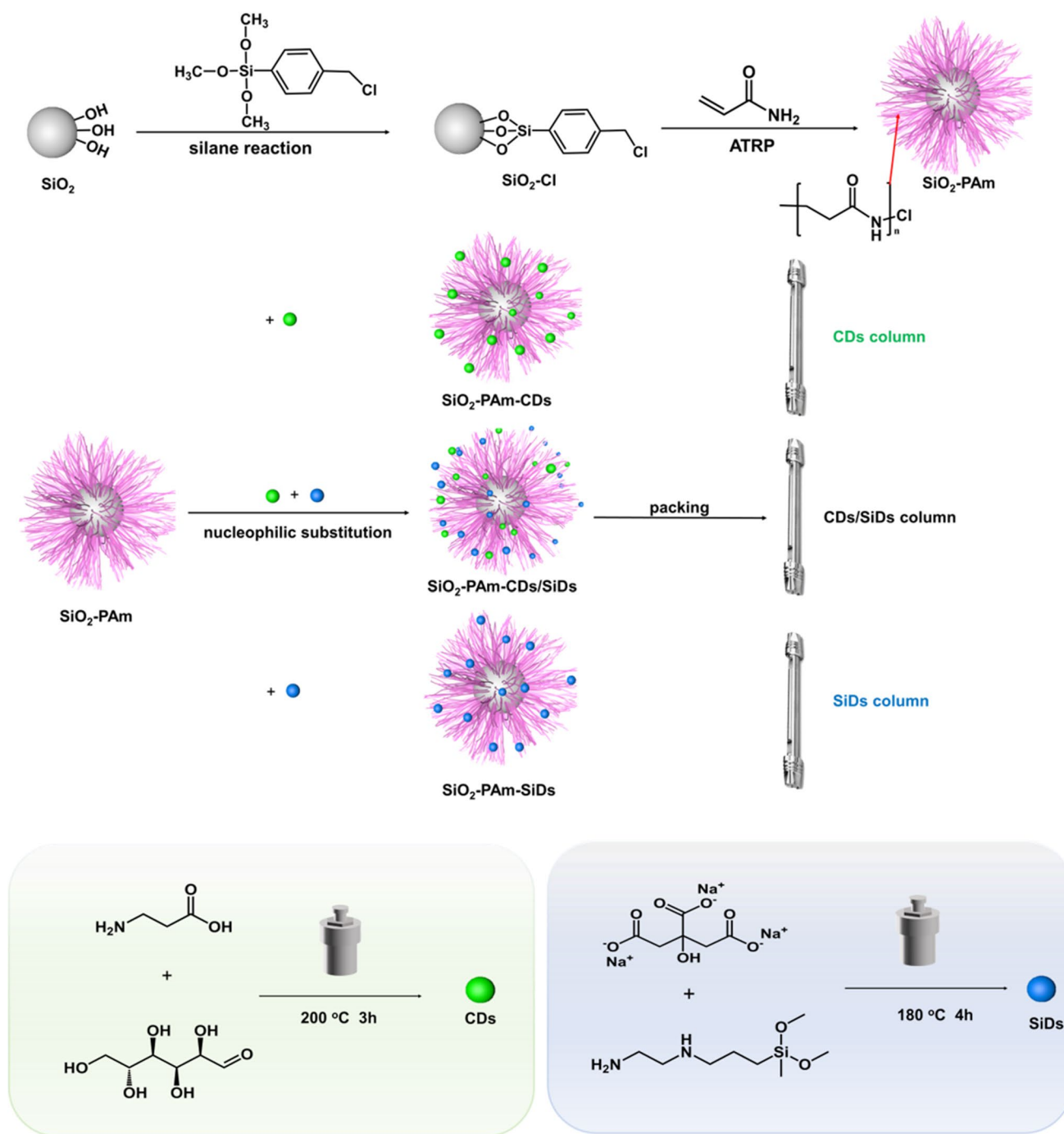
### Apparatus and testing methods

Apparatus and testing methods are displayed in the Supplementary information.

### Preparation of stationary phases

#### Fabrication of CDs and SiDs

CDs were fabricated by the one-step hydrothermal treatment. At first, glucose (1.0 g) and  $\beta$ -alanine (0.45 g) were dissolved in deionized water (30.0 mL). Then, the solution was transferred to the Teflon autoclave, and the reacted temperature was 200 °C for 3 h. After cooling to room temperature, the precipitate was removed by centrifugation, dialysis, and freeze-drying to get CDs.



**Fig. 1** Schematic demonstration for preparation of SiO<sub>2</sub>-PAm-CDs, SiO<sub>2</sub>-PAm-CDs/SiDs, and SiO<sub>2</sub>-PAm-SiDs stationary phases

SiDs were synthesized as same as the above method of CDs. Sodium citrate (0.368 g) was dissolved in deionized water (8.0 mL), and AEAPMS (2.0 mL) was added with stirring. After ultrasonic mixing, it was added to the Teflon autoclave at 180 °C for 4 h. After cooling to room temperature, the precipitate was removed by centrifugation, dialysis, and freeze-drying to obtain SiDs.

### Synthesis of SiO<sub>2</sub>-PAm-CDs/SiDs

According to our previous report [17], the ATRP initiator (SiO<sub>2</sub>-Cl) was prepared by the silane reaction, and then Am was grafted on the surface of SiO<sub>2</sub> by the one-step ATRP (the product denoted as SiO<sub>2</sub>-PAm-Cl). The specific synthesis of SiO<sub>2</sub>-Cl and SiO<sub>2</sub>-PAm-Cl are shown in the Supplementary information.

Then, the -Cl still exists at the end of PAm brushes after ATRP. The further bonding of CDs and SiDs was easily performed by nucleophilic substitution between the  $\text{-NH}_2$  in CDs/SiDs and the -Cl in  $\text{SiO}_2$ , specifically as follows:  $\text{SiO}_2$ -PAm (2.0 g) was suspended in water (40 mL), dispersed by sonication, and pyridine (0.1 mL), and CDs (0.5 g) and SiDs (0.5 g) were added, respectively. After a reaction of 65 °C for 24 h, the obtained  $\text{SiO}_2$ -PAm-CDs/SiDs stationary phases were washed with water and then dried at 50 °C. Simultaneously, the bonding of single CDs or SiDs was also performed. The synthesis procedure of  $\text{SiO}_2$ -PAm-CD and  $\text{SiO}_2$ -PAm-SiDs was the same as above, except for adding the single CDs (0.5 g) or SiDs (0.5 g) in the reaction system.

### Chromatographic column packing and testing

Slurry suspension of the stationary phase was loaded into the chromatographic column (50 × 4.6 mm I.D.) through a high-pressure pneumatic pump (CGY-100B, Beijing, China). The three stationary phases,  $\text{SiO}_2$ -PAm-CDs/SiDs,  $\text{SiO}_2$ -PAm-CDs, and  $\text{SiO}_2$ -PAm-SiDs, were respectively weighed at 0.8 g and packed into stainless steel columns (50 mm × 4.6 mm I.D.) under 45 MPa for 30 min using water as the propulsion solvent and isopropyl alcohol as the slurry solvent, obtaining three columns (denoted as CDs/SiDs column, CDs column, and SiDs column, respectively).

Next, the three columns were subsequently connected to the LC instrument, and it was rinsed and activated with methanol before routine testing. Chromatographic experiments were performed on a Shimadzu LC system (Kyoto, Japan), which consists of two LC-20ATvp pumps, a SCL-10A system controller, a UV detector, and a Class VP 5.03 chromatographic workstation. All chromatographic separations were performed using nucleic bases (uracil, adenine, cytosine, and guanine); organic acids (ferulic acid, benzoic acid, vanillic acid, and syringic acid); and  $\beta$ -agonists (terbutaline metaproterenol and dopamine hydrochloride) as test compounds, and in HILIC mode, the mobile phase was ACN/water with different volume ratios, while in PALC mode, the mobile phase was only water. The flow rate was 1.0 mL  $\text{min}^{-1}$ , the column temperature was 25 °C except for the temperature-responsive experiment, and the detection wavelength was 254 nm. Toluene was employed as the column void time ( $t_0$ ) marker in HILIC mod, and three times each measurement was replicated.

### Pretreatment of Goji berry sample

The procedures for treating the Goji berry sample were as follows: 0.2 g of Goji berry was dried in a centrifuge tube, 25.0 mL of methanol solution was added, homogenized after vortex, centrifuged at 3500 r/min, and taken out for later use. 5.0 mL of supernate was transferred to the activated

cation-exchange solid-phase extraction column, the flow rate was controlled at 1 drop/s, and after the test sample entered the adsorption layer of the extraction cartridge, 5 mL of methanol was used to rinse sequentially. Finally, it was eluted with 5 mL of ammoniated methanol and repeated. The eluent was concentrated near dryness and dissolved in a 2 mL ACN for a certain volume, detection after filtration.

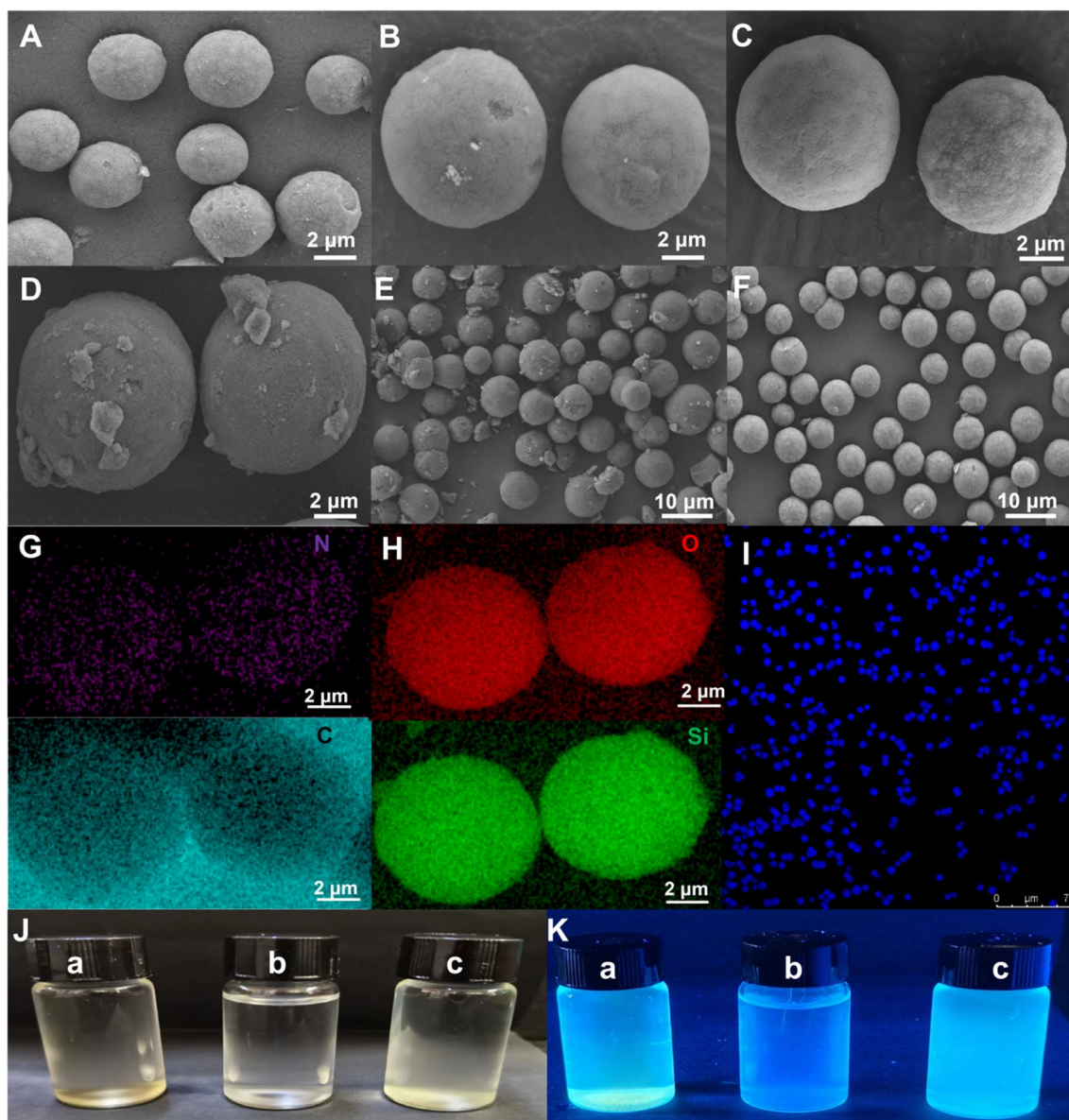
## Results and discussions

### Synthesis and characterization of $\text{SiO}_2$ -PAm-CDs/SiDs

The preparation process for the stationary phase is shown in Fig. 1. Here, a one-step hydrothermal method was used to synthesize CDs using glucose and  $\beta$ -alanine as precursors, as well as SiDs using sodium citrate and 3-(2-aminoethylamino) propyldimethoxymethyl silane as precursors, respectively. Then, benzyl chloride ( $\text{-CH}_2\text{Cl}$ ) modified silica as an ATRP initiator was synthesized by the classical silane reaction, and then the hydrophilic and temperature-sensitive monomer of Am was reasonably polymerized by ATRP to synthesize  $\text{SiO}_2$ -PAm. Since the -Cl still existed on the end of PAm chains after an ATRP reaction, the hydrophilic ligands, CDs or SiDs, were easily bonded at the terminal of PAm chains through nucleophilic substitution between Cl of  $\text{SiO}_2$  and  $\text{NH}_2$  of CDs or SiDs, forming the three stationary phases of  $\text{SiO}_2$ -PAm-CDs,  $\text{SiO}_2$ -PAm-CDs/SiDs, and  $\text{SiO}_2$ -PAm-CDs/SiDs.

For the stationary phase, the regular spherical shape and good dispersion of particles can effectively reduce the eddy current diffusion. In this regard, the morphology of stationary phases was first observed by SEM. Compared with the original  $\text{SiO}_2$  matrix (Fig. 2A), the stationary phases remained spherical and uniform in size distribution after the grafting of AM and respective modification of CDs (Fig. 2B) and SiDs (Fig. 2C). Moreover, for  $\text{SiO}_2$ -PAm-CDs/SiDs stationary phase, its SEM pictures could observe the homogeneous coating with some spots (Fig. 2D), and after modification, its regular spherical shape and good dispersion still maintained (Fig. 2E and F), and further through mapping analysis, it was found that  $\text{SiO}_2$ -PAm-CDs/SiDs contained four elements: Si, N, C, and O (Fig. 2G, H). Since CDs or SiDs are fluorescent luminous bodies, considering, fluorescence imaging of the surface chemical identity of  $\text{SiO}_2$ -PAm-CDs/SiDs was performed with CLSM. Indeed, after grafting of CDs/SiDs, the obtained  $\text{SiO}_2$ -PAm-CDs/SiDs stationary phase displayed the monodisperse spherical-shaped morphology and identified the blue-fluorescent (Fig. 2I). Under daylight conditions, CDs, SiDs, and CDs/SiDs-modified  $\text{SiO}_2$  particles possessed good solubility and dispersibility in water (Fig. 2J). Further comparing





**Fig. 2** SEM images of SiO<sub>2</sub> (A), SiO<sub>2</sub>-PAM-CDs (B), and SiO<sub>2</sub>-PAM-SiDs (C). SEM images (D–F) and the corresponding mapping images of N, C, O, Si (G and H) and fluorescence imaging (I) of

SiO<sub>2</sub>-PAM-CDs/SiDs. Under daylight irradiation photos (J) and ultra-violet irradiation (K) of SiO<sub>2</sub>-PAM-CDs (a), SiO<sub>2</sub>-PAM-SiDs (b), and SiO<sub>2</sub>-PAM-CDs/SiDs (c) in turn, respectively

their fluorescence colors under ultraviolet irradiation, clearly green, blue, and cyan fluorescence were observed for SiO<sub>2</sub>-PAM-CDs, SiO<sub>2</sub>-PAM-SiDs, and SiO<sub>2</sub>-PAM-CDs/SiDs (Fig. 2K), respectively.

Chemical elements of the prepared SiO<sub>2</sub>-based stationary phases were analyzed by EA, FI-TR, and XPS in turn. EA data are displayed in Table 1; clearly, compared to SiO<sub>2</sub>-Cl, SiO<sub>2</sub>-PAM showed a significant increase in C, H, and N contents, demonstrating the successful polymerization of Am onto SiO<sub>2</sub>. After further modification of CDs, CDs/SiDs, and SiDs, different increases in the elemental content of C and N were observed for SiO<sub>2</sub>-PAM-CDs (C, 11.81%; N,

**Table 1** Elemental analysis of SiO<sub>2</sub>-Cl, SiO<sub>2</sub>-PAM, SiO<sub>2</sub>-PAM-CDs, SiO<sub>2</sub>-PAM-CDs/SiDs, and SiO<sub>2</sub>-PAM-SiDs

Materials	Elemental analysis (%)		
	C%	H%	N%
SiO <sub>2</sub> -Cl	0.79	0.69	0.00
SiO <sub>2</sub> -PAM	3.54	1.02	1.19
SiO <sub>2</sub> -PAM-CDs	11.81	1.78	4.47
SiO <sub>2</sub> -PAM-CDs/SiDs	18.94	2.99	5.19
SiO <sub>2</sub> -PAM-SiDs	20.31	3.18	5.54

4.47%), SiO<sub>2</sub>-PAm-CDs/SiDs (C, 18.94%; N, 5.19%), and SiO<sub>2</sub>-PAm-SiDs (C, 20.93%; N, 5.54%), compared with C (3.54%) and N (1.19%) of SiO<sub>2</sub>-PAm, which also indicated the successful integration of CDs or SiDs. Obviously, their C and N contents were different, which could predict the surface charge differences for those stationary phases. After testing their charges by zeta potential, as shown in Fig. S1, SiO<sub>2</sub>-PAm-CDs and SiO<sub>2</sub>-PAm-CDs/SiDs had the negative charge (−14.1 and −1.86 eV), while for SiO<sub>2</sub>-PAm-SiDs, with the highest N contents among those stationary phases, it had a positive charge (1.23 eV), which was consistent with the N differences by EA. Furthermore, the surface chemical composition of those materials was measured by XPS. As can be seen from Fig. S2A, those peaks, Si 2p at 101.1 eV, C 1 s at 285.0 eV, N 1 s at 398.4 eV, and O 1 s at 535.1 eV, all appeared in the XPS spectrum, which is attributed to the presence of Am, CDs, and SiDs in those SiO<sub>2</sub> stationary phases. Then, FT-IR was used to characterize the functional groups, and the FT-IR spectra of SiO<sub>2</sub> stationary phases were shown in Fig. S2B. It was found that peaks at 665 and 801 cm<sup>−1</sup> were assigned to the stretching vibration of C–Cl and Si–O, which indicated the successful silane reaction occurred to obtain SiO<sub>2</sub>-Cl. The peak at 2971 cm<sup>−1</sup> was representative of the C–H stretch vibration, at 1160 cm<sup>−1</sup> was due to the stretching vibration of C–N, and at 1690 cm<sup>−1</sup> belonged to C=O stretching, which was attributed to graft Am. Besides, the obviously enhanced peak intensity of Si–O at 801 cm<sup>−1</sup> was simultaneously observed in SiO<sub>2</sub>-PAm-CDs/SiD and SiO<sub>2</sub>-PAm-SiDs stationary phases because of successfully bonding SiDs.

### Chromatographic performances of the developed columns

Here, the three polar stationary phases were simply constructed by firstly grafting hydrophilic chains and then bonding SiDs or CDs on the terminal chains. The grafting of PAm chains could endow the stationary phase with the polar amide groups, and then the introduction of SiDs or CDs with a biocompatible property could further enhance the hydrophilic strength of stationary phases to improve the HILIC retention. In the experiment, the three stationary phases, SiO<sub>2</sub>-PAm-CDs, SiO<sub>2</sub>-PAm-CDs/SiDs, and SiO<sub>2</sub>-PAm-SiDs, were packed to be named as CDs column, CDs/SiDs column, and SiDs column, and a diversity of hydrophilic test solutes, including nucleic bases, organic acids, and β-agonists, were engaged to probe separation efficiency, and their structures with log *p* and *p**k*<sub>a</sub> values are listed in Fig. S3.

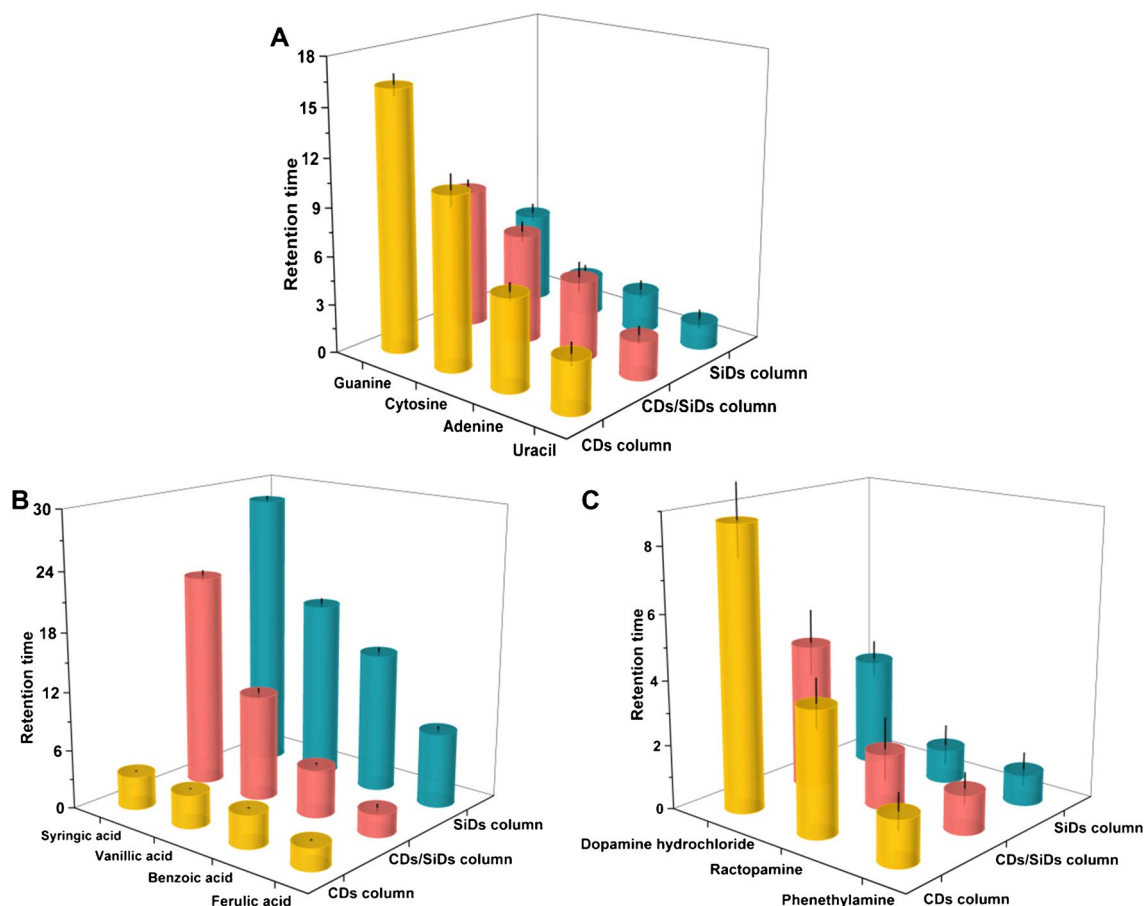
### Retention behavior of test solutes on the three columns

According to our devised stationary phases, the bonding of hydrophilic ligands (PAm and CDs/SiDs) theoretically

endows the packed columns with HILIC retention. The most known feature of HILIC is that the retention of solute decreases as the water content of the strong eluent increases in the mobile phase [19]. When the content of water in the mobile phase was adjusted in the range of 5–40% to investigate retention clearly (see Fig. S4), all the retention of nucleic bases, organic acids, and β-agonists on the three columns decreased as the water content increased, obeying a typical HILIC retention. Further comparing the retention strength on the three columns using the same separation conditions, as displayed in Fig. 3, the retention of nucleic bases and β-agonists was both the strongest on the CDs column and the weakest on the SiDs column, but the retention of organic acids on both columns was just opposite (Fig. 3A, C). On the CDs/SiDs column, the retention strengths of all test solutes were between the CDs and SiDs columns (Fig. 3B). These results verify the relatively strong retention of basic solutes in the CDs column and that of acidic solutes in the SiDs column. Expectedly, these ranges in the retention behaviors were related to the differences in the charge properties of the stationary phase because of the structural CDs or SiDs with the numeric differences of amine, hydroxy, and carboxylic groups, resulting in the difference in surface charge of the stationary phase. Indeed, as stated above in Fig. S1, the zeta potential of CDs column-packed SiO<sub>2</sub>-PAm-CDs stationary phase had a negative charge (−14.1 eV), making the electrostatic attraction to basic solutes. SiDs column-packed SiO<sub>2</sub>-PAm-SiDs stationary phase had a positive charge (1.23 eV), correspondingly; it imposed the electrostatic attraction to acidic solutes. Thus, it is rational that the retention of nucleic bases and β-agonists was strong in the CDs column while that of organic acids was weak, whereas the SiDs column exhibited a reversed retention trend compared to the CDs column.

### Retention mechanism on the three columns

As stated above, the differences in retention strength could be due to the numeric differences of the amine, hydroxy, and carboxylic groups of the CDs, SiDs, and CDs/SiDs units, making the different electrostatic forces toward acidic or basic solutes. So next, the ionic interaction was examined by the effect of salt in the mobile phase on retention. It has been proven that more ions would penetrate into the water-enriched layer of the stationary phase surface when increasing salt concentration, resulting in stronger hydrophilic retention [20]. Indeed, the highly polar nucleic bases followed this rule; their retention on the three columns exhibited stronger retention as the salt concentration increased (Fig. S5A–C). Similarly, the retention times of organic acids in the CDs column and β-agonists in the SiDs column were both enhanced with the higher buffer salt concentration (Fig. S5D, I). But the retention of β-agonists in the CDs



**Fig. 3** Comparison with the retention strength for nucleic bases (**A**), organic acids (**B**), and  $\beta$ -agonists (**C**) in the CDs column, CDs/SiDs column, and SiDs column. Mobile phase: ACN/water (90/10, v/v)

for **A** and ACN/20 mM  $\text{NH}_4\text{COOH}$  (90/10, v/v) for **B** and **C**; detection wavelength: 254 nm. Column temperature: 25 °C. Flow rate: 1.0 mL  $\text{min}^{-1}$

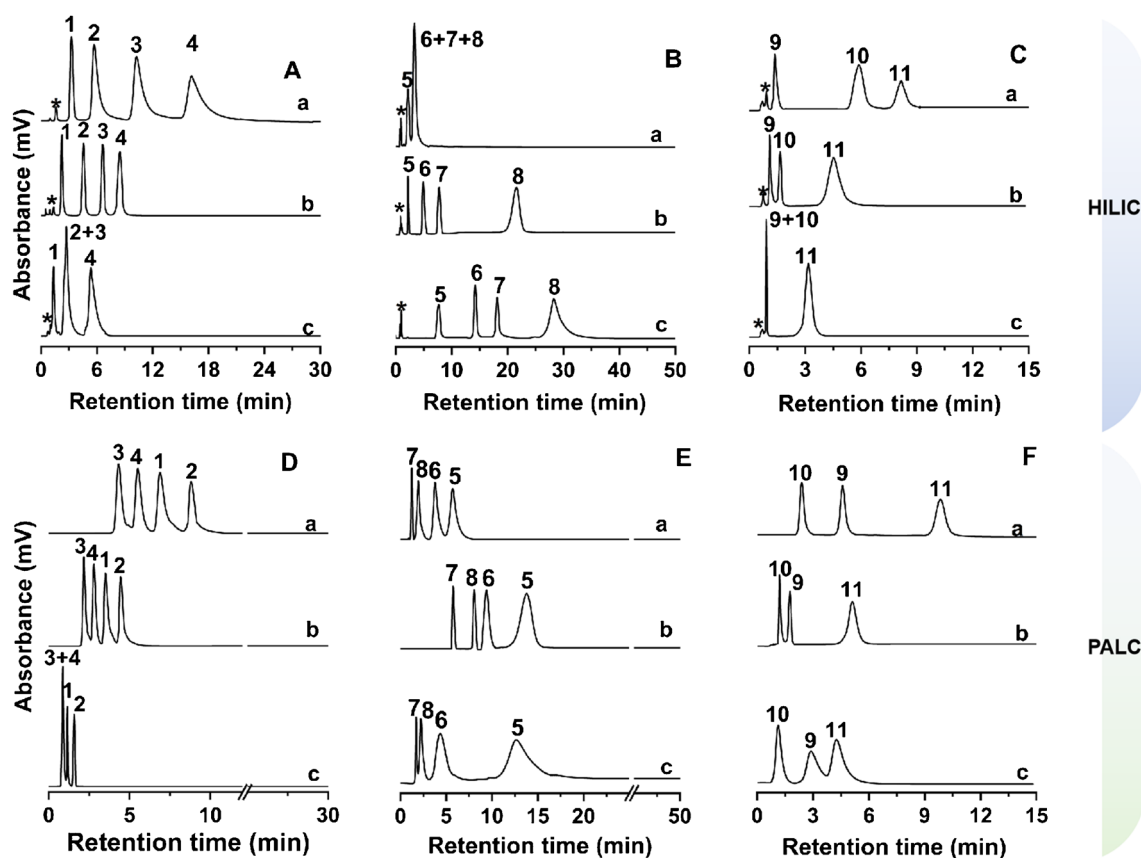
column and that of organic acids in the SiDs column both decreased when increasing salt concentration (Fig. S5G, F), indicating that the retention of  $\beta$ -agonists in the CDs column and organic acids in the SiDs column was dominated by electrostatic interaction. Whereas for the CDs/SiDs column, the retention of solutes had a different effect by salt content (Fig. S5B, E, and H); this could probably be ascribed to provide the multiple interactions, such as electrostatic, hydrophilic forces, in separation.

Always, the three models, partitioning and adsorption mechanism or a combination of partitioning and adsorption mechanisms, are often used to elucidate behaviors of HILIC retention [21]; the corresponding relationship between the retention factor ( $k$ ) of solute and the concentration of water ( $\varphi$ ) is established by the partitioning model equation (Eq. S1) [22], the adsorption model equation (Eq. S2) [23], and the combination of partitioning and adsorption model equation (Eq. S3) [24], respectively. Here, take the CDs/SiDs column as an example. The fitting results of the above three models for test solutes at the different mobile phase

compositions ( $\varphi$  is between 0.05 ~ 0.3 for nucleic bases, 0.1 ~ 0.4 for organic acids, and 0.05 ~ 0.1 for  $\beta$ -agonists) are illustrated in Fig. S6; the relationship was well fitted to Eq. S3 with coefficients of  $R^2 \geq 0.99$  for those solutes, confirming that a combination of the partitioning and adsorption mechanisms did obey in retention. From the structure of the  $\text{SiO}_2$ -PAm-CDs/SiDs stationary phase and solutes, the hydrophilic and electrostatic interactions between the tested solutes and PAm-CDs/SiDs modified  $\text{SiO}_2$  might dominate the retention, so the mixed retention mechanisms are deemed reasonable.

### Separation performance in HILIC and PALC mode

To test their separation performance, the columns were first applied to separate nucleic bases, organic acids, and  $\beta$ -agonists under HILIC mode (ACN-rich mobile phase). From these results (see Fig. 4A–C), it is evident that there were three major differences in the separation performances of those columns: (i) the order of retention strength for



**Fig. 4** Separation chromatograms of nucleic bases (**A, D**), organic acids (**B, E**), and  $\beta$ -agonists (**C, F**) by CDs column (a), CDs/SiDs column (b), and SiDs column (c) on HILIC mode and PALC mode. Analytes: 1, uracil; 2, adenine; 3, cytosine; 4, guanine; 5, ferulic acid; 6, benzoic acid; 7, vanillic acid; 8, syringic acid; 9, terbutaline; 10,

metaproterenol; 11, dopamine hydrochloride. Mobile phase: ACN/water (90/10, v/v) for **A** and ACN/20 mM  $\text{NH}_4\text{COOH}$  (90/10, v/v) for **B** and **C**; 100% water for **D** and 100% of 20 mM  $\text{NH}_4\text{COOH}$  solution for **E, F**. UV detection at 254 nm. Flow rate:  $1.0 \text{ mL min}^{-1}$ .

nucleic bases and  $\beta$ -agonists followed, CDs column > CDs/SiDs column > SiDs column, and those basic solutes could be separated well by CDs and CDs/SiDs columns; (ii) separation time of organic acids was the shortest on CDs column within 5 min while that was observed the longest on SiDs column within 30 min, displaying the baseline separation on SiDs column; (iii) selectivity factor ( $\alpha$ ) of nucleic bases, organic acids, and  $\beta$ -agonists was compared in Table S1. Clearly, the CDs/SiDs column had a moderate  $\alpha$ , with which the separation of cytosine/guanine, terbutaline/metaproterenol, and metaproterenol/dopamine was efficient. By the comparison of those columns, the CDs/SiDs column exhibited moderate retention while having high separation efficiency for all solutes under the short separation time. This result implied that the coexistence of CDs and SiDs could boost HILIC selectivity, which could be regarded as proof of the cooperation of CDs and SiDs.

Apparently, HILIC separation should take an ACN-rich mobile phase. PALC separation implements separation under a water-rich mobile phase using no or only a small

amount of organic solvent, so it is extensively encouraged to be used for economic reasons and pollution prevention [25]. Here, a diversity of polar solutes, nucleic bases, organic acids, and  $\beta$ -agonists was tentatively separated with pure water or 100% of 20 mM  $\text{NH}_4\text{COOH}$ ; the separation results are shown in Fig. 4D–F. Obviously, by comparing to HILIC results (see Fig. 4A–C), the almost same separation capacity for those solutes in PALC mode was gained as HILIC, and the peak shapes hardly changed. Taking CDs/SiDs as a representative column, the number of theoretical plate ( $N/m$ ) of nucleic bases, organic acids, and  $\beta$ -agonists are displayed in Table S2; the highest plate numbers reached 19120  $N/m$  in HILIC and 27085  $N/m$  in PALC, respectively; this demonstrated the good column efficiency in both HILIC and PALC separation. However, the chromatograms under PALC illustrated the differences in separation selectivity and elution orders of solutes in water-rich mobile phases. Researchers have reported that the mobile phase consists of a high proportion of water in PALC, reversing the polarity surface of the stationary phase to non-polar [26]. Hence,



the general elution orders for those solutes should follow their hydrophobicity in the water-rich chromatography, correspondingly; the lower polarity of solutes changed to retain strongly. As a result, on CDs, CDs/SiDs, and SiDs columns, the almost reverse elution orders of nucleic bases, organic acids, and  $\beta$ -agonists were observed under PALC retention by comparing to that of HILIC. Similarly, under PALC, the test solutes also had varied separation selectivity on those columns (Table S3), but their retention displayed a similar strength as HILIC. In specific, the stronger retention of nucleic bases and  $\beta$ -agonists was both found on the CDs column and that of organic acids shown on the SiDs column. This also ascribed that the electrostatic interaction imposed on charge solutes when separation occurred in an aqueous solution, elevating their retention on the corresponding columns. Also, the CDs/SiDs column with the proper hydrophobic and electrostatic forces had good selectivity to separate nucleic bases using 100% water and organic acids as well as  $\beta$ -agonists using 20 mM of  $\text{NH}_4\text{COOH}$  as mobile phases (see curve b of Fig. 4). Those results manifested that our devised PALC had an appropriate alternative to HILIC for the separation of those polar compounds.

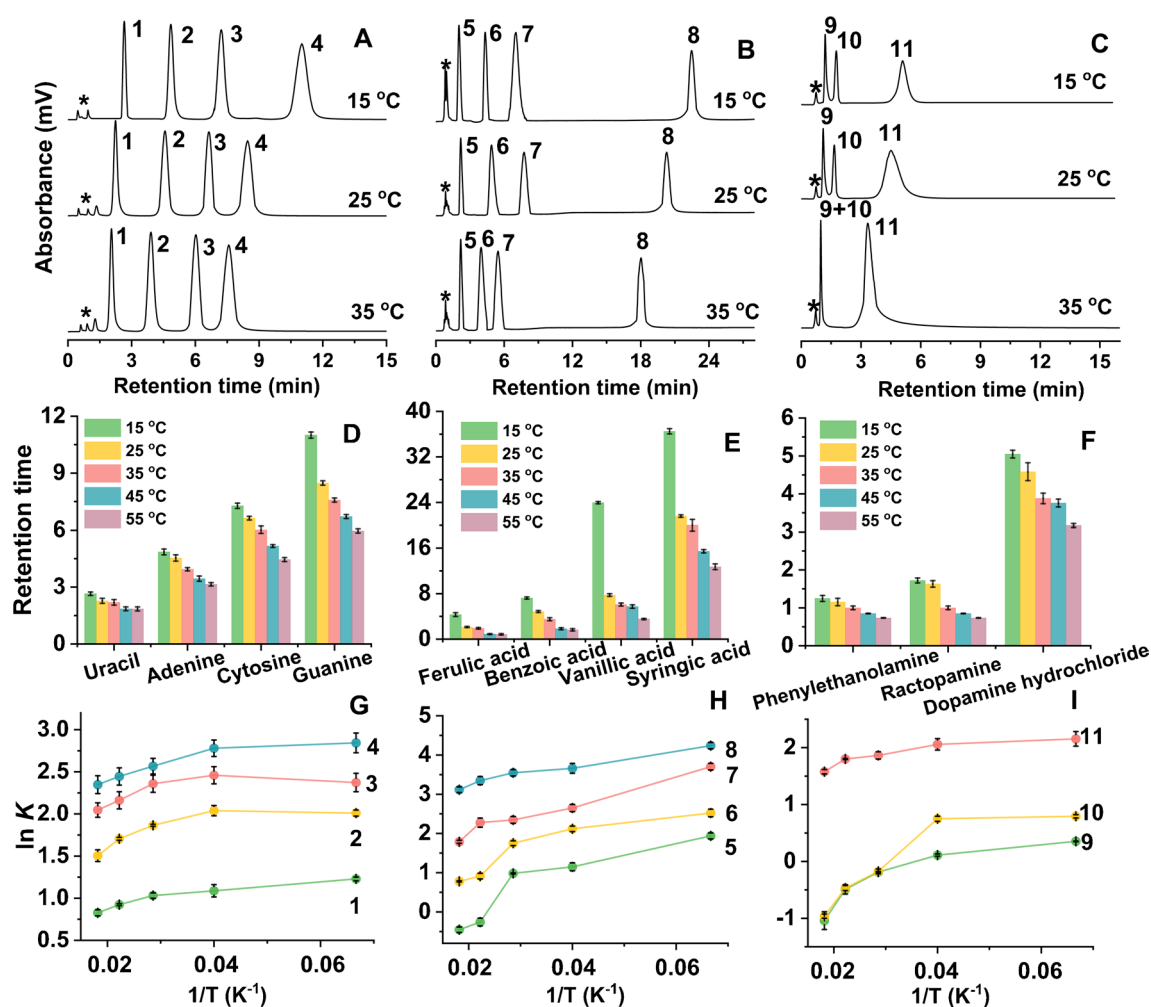
Besides, the advantages of our developed columns were highlighted by comparison with the  $\text{SiO}_2$ -PAm column, as shown in Fig. S7; under both the HILIC and PALC modes, it is clear that the  $\text{SiO}_2$ -PAm column showed poor separation selectivity for nucleic bases, organic acids, as well as  $\beta$ -agonists. Especially under PALC mode, by using 100% water separation, the solute retention strength showed much weakness on the  $\text{SiO}_2$ -PAm column than that on CDs, CDs/SiD, or SiDs columns. This ascribed that the presence of CDs or SiDs could promote to reverse the polarity surface of the stationary phase to non-polar when the mobile phase consisted of a high proportion of water, enhancing retention. This comparison suggested that combining PAm and CDs/SiDs could enhance the separation selectivity of the PALC stationary phase.

### Effect of column temperature on retention

Column temperature affects retention since the variation in temperature could change the diffusion coefficients and interactions between the solute and stationary phase. Here, under ACN-rich HILIC mode, the retention of nucleic bases, organic acids, and  $\beta$ -agonists on the representative CDs/SiDs column reduced with increasing column temperatures when the column temperature was fixed at 15, 25, and 35 °C (Fig. 5A–C). The effect of column temperature on retention was investigated in detail from 15 to 55 °C with an interval of 10 °C, but the retention of all solutes still showed a decreasing trend as the column temperature increased (Fig. 5D–F). Theoretically, the higher temperature can decrease the viscosity of the mobile phase to accelerate

the diffusivity and solubility of solutes in the mobile phase, making a rapid mass transfer rate. Hence, under HILIC mode, the weakened retention with the enhanced column temperature was rational. The van't Hoff equation (Eq. S4) is used to explain the effect of temperature on retention [27]. In general, the linear plots between  $\ln k$  and  $1/T$  can prove that the influence of temperature is mainly occupied by the partitioning interaction [28]. But as illustrated in Fig. 5G–I, the variations of nucleic bases, organic acids, and  $\beta$ -agonists were all nonlinear on the CDs/SiDs column. This curvature in the van't Hoff plot further confirmed the multiple mechanisms (e.g., hydrophilic and electrostatic forces) to retention.

In PALC just using 100% water as the mobile phase, the single component of the mobile phase is essentially constant; it is impossible to modulate the composition of the mobile phase to adjust separation selectivity. So, under PALC mode, changing column temperature is an effective way to adjust separation. The separation chromatograms at temperatures of 25, 35, and 45 °C for nucleic bases, organic acids, and  $\beta$ -agonists were shown in Fig. 6A–C, respectively. It was observed that their resolution was significantly changed by modulating column temperature, and the retention was stronger at 35 °C than that at 25 and 45 °C. Further investigation of temperatures from 15 to 55 °C found that their retention displayed an increasing trend below 35 °C and then declined as the temperature above 35 °C enhanced (Fig. 6D–F). Meanwhile,  $\ln k$  against  $1/T$  plots also showed non-linear relationships (Fig. 6G–I). This could be ascribed that the existing PAm chains in the stationary phase, which is a classical temperature-sensitive polymer with an upper critical solution temperature (UCST) at about 32–35 °C, are capable of endowing the stationary phase with temperature-sensitive properties besides HILIC retention. Theoretically, the PAm chain has hydrophobicity in its collapse state at a temperature below UCST and becomes hydrophilicity in its swollen state at a temperature above UCST [29]. Hence, under pure water solution, CDs/SiDs column packed temperature-sensitive  $\text{SiO}_2$ -PAm-CDs/SiDs stationary phase could adjust the hydrophobic-hydrophilic surface properties of the stationary phase by modulating column temperatures, achieving the alteration of separation. Herein, the following reason is thereby that the resulting plotting of the effect of temperature on retention was consistent with the temperature-sensitive characteristics. Indeed, below UCST, the collapse PAm was in the proceeds with increasing column temperatures, enhancing the hydrophobic strength. Under PALC separation, the hydrophobic interaction was the main force to induce retention; as a result, a large enhancement of  $k$  was all observed below 35 °C of UCST for nucleic bases, organic acids, and  $\beta$ -agonists. When continuously increasing column temperature above 35 °C, PAm chains changed to a swollen form to expose hydrophilic properties; this made the hydrophobic interaction weaken the retention at



**Fig. 5** Separation (A–C), the effect of column temperature on retention (D–F), and plots of  $\ln k$  vs  $1/T$  (G–I) of nucleic bases, organic acids, and  $\beta$ -agonists for SiO<sub>2</sub>-PAm-CQDs/SiQDs column. Peaks: 1, uracil; 2, adenine; 3, cytosine; 4, guanine; 5, ferulic acid; 6, benzoic acid; 7, vanillic acid; 8, syringic acid; 9, terbutaline; 10, metaproterenol; 11, dopamine hydrochloride. Mobile phase: ACN-H<sub>2</sub>O containing 20 mM NH<sub>4</sub>COOH (90/10, v/v). UV detection at 254 nm. Flow rate: 1.0 mL min<sup>-1</sup>

acid; 7, vanillic acid; 8, syringic acid; 9, terbutaline; 10, metaproterenol; 11, dopamine hydrochloride. Mobile phase: ACN-H<sub>2</sub>O containing 20 mM NH<sub>4</sub>COOH (90/10, v/v). UV detection at 254 nm. Flow rate: 1.0 mL min<sup>-1</sup>

a temperature above 35 °C (Fig. 6G–I). As a result, a combination of temperature-response and pure water separation could promote the efficiency of our devised green PALC columns.

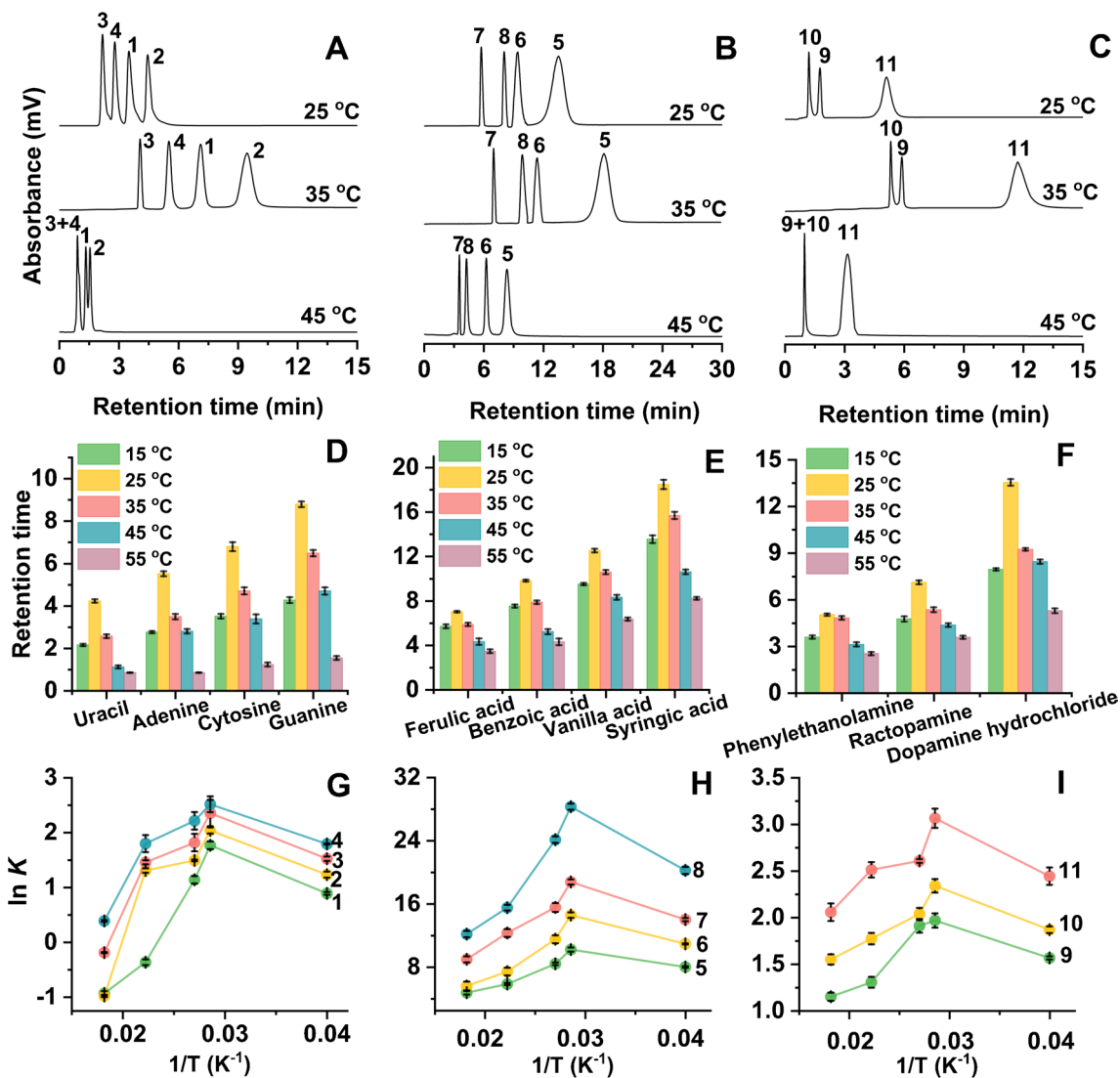
### Stability of the SiO<sub>2</sub>-PAm-CDs/SiDs column

The stability of the PALC stationary phase is extremely important because the column efficiency will be seriously decreased after the mobile phase is used as water for a long time [30]. So, for the practical application under pure water separation, longevity is a critical factor to be concern. Here, the selection of highly polar nucleic bases as probes and CDs/SiDs column was used to check the stability. The day-time reproducibility was studied in 100% water as a mobile phase with continuous injection 6 times per day and repeated operation for 3 days, as seen in Fig. S8. The retention times

and peak shapes were both hardly changed by comparing with the initial injection, and their variations of retention time were below 0.07–1.02% RSDs ( $n=18$ ). Moreover, the CDs/SiDs column had not observed the losses of column efficiencies. In this regard, the combing of CDs/SiDs and hydrophilic polymers for the devised PALC stationary phase had superior robustness even using 100% water, being used as a wide and reliable separation medium.

### Quantitative determination of betaine in Goji berry

In the practical application, betaine is a main content of Goji berry, which has been used clinically for nearly 50 years. Recently, for analysis of betaine, the most common usage of LC columns contain strong-cation exchange column using the complex component of mobile phase (ACN-potassium dihydrogen phosphate-phosphoric acid), and HILIC types of



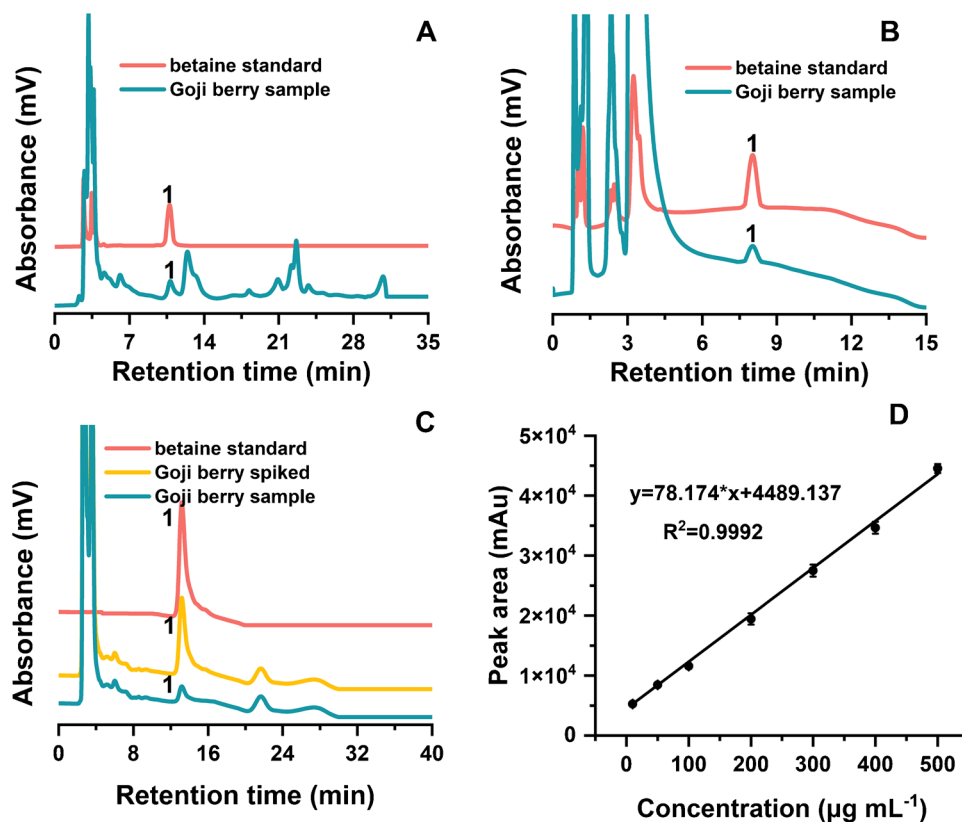
**Fig. 6** Separation (A–C), the effect of column temperature on retention (D–F), and plots of  $\ln k$  vs  $1/T$  (G–I) of nucleic bases, organic acids, and  $\beta$ -agonists for CDs/SiDs column. Peaks: 1, uracil; 2, adenine; 3, cytosine; 4, guanine; 5, ferulic acid; 6, benzoic acid; 7,

vanillic acid; 8, syringic acid; 9, terbutaline; 10, metaproterenol; 11, dopamine hydrochloride. Mobile phase: 100% H<sub>2</sub>O for A and 20 mM NH<sub>4</sub>COOH for B and C. UV detection at 254 nm. Flow rate: 1.0 mL min<sup>-1</sup>.

NH<sub>2</sub>, Am, and Diol columns, which are respectively packed amine or amide or diol modified SiO<sub>2</sub> as stationary phase using high ACN-rich (over 85%) of mobile phase [31]. In this work, our packed CDs/SiDs column was utilized to establish a LC method for monitoring betaine from Goji berry. After routinely handling by being smashed, shaken, and centrifuged for Goji berry samples, the obtained solution was processed by filter membrane for LC analysis, and two commercial NH<sub>2</sub> and Diol columns as control were simultaneously implemented. The representative chromatograms by those columns are displayed in Fig. 7. Under ACN/water (85%/15%, v/v) as mobile phase, the peak of betaine was detected by the NH<sub>2</sub> column, but clearly, there were a lot of interference peaks nearby, having invalid separation

with the matrix of Goji berry (Fig. 7A). For Diol column, the better result was obtained because of the effective suppression of interference compared to NH<sub>2</sub> column, noting that this effective separation needs not only ACN-rich of mobile phase but also gradient elution of complex separation process (Fig. 7B). It is important to mention that efficient separation could be achieved in our CDs/SiDs column. Under the PALC mode (using 100% water as separation conditions), betaine was clearly detected. Meanwhile, the interference peaks were efficiently inhibited (Fig. 7C). Subsequently, based on matrix addition methods, the standard curve of betaine was gained with good correlation coefficients (Fig. 7D) and also had high recoveries and low RSDs in Goji berry (Table S4), obtaining 7.3 mg g<sup>-1</sup> of betaine

**Fig. 7** Chromatograms of betaine detected from Goji berry by  $\text{NH}_2$  column (A), Diol column (B), and CDs/SiDs column (C), as well as the standard curve of betaine by CDs/SiDs column (D). Mobile phase, ACN-water (85/15, v/v) for  $\text{NH}_2$  column; gradient elution: 0–2 min ACN/water (95/5–90/10), 2–3 min ACN/water (90/10–70/30), 3–4 min ACN/water (70/30–50/50), 4–9 min ACN/water (50/50), 9–10 min ACN/water (50/50–95/15), 10–15 min ACN/water (95/15) for diol column; 100 water for CDs/SiDs column; flow rate,  $1.0 \text{ mL min}^{-1}$  for  $\text{NH}_2$  column and diol column,  $0.7 \text{ mL min}^{-1}$  for CDs/SiDs column; detection wavelength, 195 nm; temperature,  $30^\circ \text{C}$



content in Goji berry. In conclusion, the CDs/SiDs column could separate betaine from Goji berry just using pure water and isocratic elution, showing the application potential in the field of active ingredient analysis by green separation.

## Conclusion

This paper described the synthesis, characterization, and chromatographic evaluation of the hydrophilic PAM chains grafted  $\text{SiO}_2$  with post modification of CDs, CDs/SiDs, and SiDs. The developed stationary phases successfully separated the various types of polar solutes, such as nucleic bases,  $\beta$ -agonist, and organic acids when using ACN-rich as the mobile phase, and most importantly, also exhibited good separation in PALC mode by using 100% water solution as mobile phase. Besides, the grafting of PAM endowed the developed PALC stationary phases with temperature-sensitivity chromatographic characteristic. So, combining the temperature-sensitive property and pure water separation conditions, the separation selectivity of our devised stationary phases could be efficiently promoted and adjusted by simply changing the column temperature. As a result, when the developed CDs/SiDs column was applied to analyze betaine in Goji berry, it had superior ability in detection and quantification by

comparing with the commercial HILIC columns. What is more important, our separation for Goji berry just used pure water as the mobile phase, while the commercial  $\text{NH}_2$  column was performed using the ACN-rich as the mobile phase and the Diol column needed the complex gradient elution to implement separation. Assuredly, those positive results from our proposed preparation strategy of the PALC stationary phase, manifesting the combination of CDs/SiDs and hydrophilic polymers, lift high level to develop green separation of the stationary phase.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00604-024-06575-y>.

**Author contribution** B: Conceptualization (lead); data curation (lead); methodology (lead); project administration (lead); supervision (supporting); writing-review and editing (lead). T and L: Methodology (equal); writing-original draft (equal); Investigation (equal); methodology (equal); software (equal). L: Formal analysis (equal); software (equal). Z: Investigation (equal); Validation (equal). G: Data curation.

**Funding** This work was supported by the Nature Science Foundation of Ningxia (no. 2023AAC03259), the Youth Talent Cultivation Project of North Minzu University (no. 2021KYQD30), the National Natural Science Foundation of China (no. 22064013 and 52263021), and the University level key research project of North Minzu University (no. 2023ZRLG19).

**Data availability** No datasets were generated or analysed during the current study.



## Declarations

**Ethical approval** This research did not involve human or animal samples.

**Competing interests** The authors declare no competing interests.

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