Supporting information for

Facile Synthesis of Spherical Covalent Organic Frameworks as Stationary Phases for Short-Column Liquid Chromatography

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EXPERIMENTAL SECTION

Materials and Instrumentation. All chemicals and reagents used were analytical grade or better, which were used without further purification. 2,5-divinylterephthalaldehyde (DVA) and 1,3,5-Tris(4-aminophenyl) benzene (TAPB) were purchased from Jilin Yanshen Technology, Ltd. (Jilin, China). Tetrahydrofuran (THF), ethanol (EtOH), acetonitrile (ACN), and methanol (MeOH) were obtained from Sinopharm Chemical Reagent, Co., Ltd (Shanghai, China). Iodoracetamide (IAA), 1,4-dithiothreitol (DTT), bovine serum albumin (BSA), and TPCK-treated trypsin were bought from Sigma-Aldrich (St. Louis, MO). All other reagents and analytes used in the ultrahigh-performance liquid chromatography (UPLC) were provided by Aladdin Chemistry Co. Ltd. (China). The deionized water used in the current experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA, U.S.A.).

Preparation of Tryptic Digestion of BSA.1 mg of BSA was first dissolved in 100 μL NH_4HCO_3 solution (50 mM, pH 8.3), denatured at 56 ℃ for 15 min. After being reduced by 200 μL DTT (100 mM) at 56 ℃ for 1h, the protein solution was subsequently alkylated by 200 μL IAA (100 mM) at 37 ℃ for 30 min in the dark. Finally, the obtained solution was diluted by NH_4HCO_3 solution to 1 ml and digested by trypsin (50:1, w/w) at 37 ℃ for 16 h. The resulting tryptic digest was stored at -20 ℃ for further use.

Characterization. Field emission scanning electron microscopes (SEM) images were obtained by FEI Nova NanoSEM 230 microscope equipment. Transmission Electron Microscopy (TEM) images were acquired on Hitachi HT7700 operated at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) data were recorded on a NanoPlus3 nanoparticle size and zeta potential analyzer using the ethanol as the dispersion solution. Fourier transform infrared (FT-IR) spectra of the solid samples were taken on a BD FACSCanto (TM) Ⅱ spectrometer. Powder X-ray diffraction (PXRD) was carried out on a CEM DY5261/Xpert3 X-ray diffractometer, and the corresponding data were collected in the range of 2θ = 2-30° at a scan rate of 2° min-1. N2 adsorption-desorption isotherms were measured on a Micromeritics ASAP 2020

automatic volumetric instrument at 77 K. The samples were degassed at 120 °C for 8 h under vacuum before measurement. The surface areas were calculated from the adsorption data using Brunauer-Emmett-Teller (BET) model. The pore-size-distribution curves were obtained using the non-localized density functional theory (NLDFT). Thermogravimetric analysis (TGA) was carried out on a STA449C/6/G analyzer from 30 to 1000 °C under Argon atmosphere with a ramp rate of 10 ºC min–1. The water contact angle (CA) was measured on a JC2000C machine with 3 μL of a water drop (Powereach, Shanghai, China). The X-ray photoelectron spectra (XPS) were analyzed by a Thermo Scientific ESCALAB 25 0.

UPLC experiments were carried out on a Waters ACQUITY UPLC H-class system (Waters, USA). C18 packed column (30 mm × 2.1 mm i.d., 1.8 μm) was purchased from Welch Materials Co., Ltd (Zhejiang, China). The concentration of all analytes was 100 mg/L, and the injection volume in chromatographic separation was 3 μL. The packed column was prepared with HY-HPLC-M (Hydrosys, China).

Synthesis of Spherical Covalent Organic Frameworks. DVA (132 mg) and TAPB (168 mg) were added into 60 mL ACN in a 100 mL glass bottle at first. Subsequently, different volumes of 12 M HAc (2.4, 4.8, 7.2, and 8.4 mL), as the catalyst for synthesis, were injected into the above-mentioned mixture, respectively. Afterward, the bottles were vigorously shaken for 20 s and then placed at room temperature to react for 48 h. The pale-yellow products were washed with ACN and EtOH four times, respectively. Finally, the yellow S_{COFs} were dried at 60 °C under vacuum for 12 h.

Preparation of the Spherical Covalent Organic Frameworks Packed Columns.

120 mg of S_{COFs} was dispersed in a centrifuge tube with 10 mL acetonitrile and vigorously ultrasonicated for 10 min. The as-prepared suspension was then poured into a UPLC column (30 mm × 2.1 mm i.d.) under 5500 psi for 40 min using methanol as

a propulsion solvent. After packing, the S_{COFs} packed columns were flushed with MeOH at a flow rate of 0.2 mL min^{-1} for 24 h using a high-pressure pump.

Calculation of the swelling tendency and thermodynamic parameters.

The swelling tendency of S_{COFs} packed columns was investigated through swelling propensity (SP) factor. SP factor in ACN can be calculated as:

$$SP = \frac{P/\eta(ACN) - P/\eta(H2O)}{P/\eta(H2O)} \quad (1)$$

where P is defined as the pressure drop of the column and η means the solvent viscosity.

The enthalpy change (ΔH) and entropy change (ΔS) for the transfer of the analyte between the mobile phase and the prepared S_{COFs} stationary phase were calculated according to the van't Hoff equation.

$$\ln k = -\Delta H/(RT) + \Delta S/R + \ln \Phi \quad (2)$$

where k is retention factor, R is gas constant, T is absolute temperature, and Φ is the phase ratio, which is defined as the volume ratio of the stationary phase (Vs) to the mobile phase (Vm).

$$k = (t - t_0)/t_0 \quad (3)$$

where t is the retention time, t_0 is the column void time which was determined by injecting a small plug of thiourea and recording the perturbation signal.

$$Vs = V_{Col} - V_0 \quad (4)$$

$$V_0 = t_0 \times F \quad (5)$$

where V_{Col} is the geometrical volume of the column, and F the flow rate of the mobile phase.

$$Rs = 2(t_{R2}-t_{R1})/(w_1+w_2) \quad (6)$$

where t_{R1} and t_{R2} are the retention times and w_1 and w_2 are the peak widths of the first and second eluted analytes, respectively.

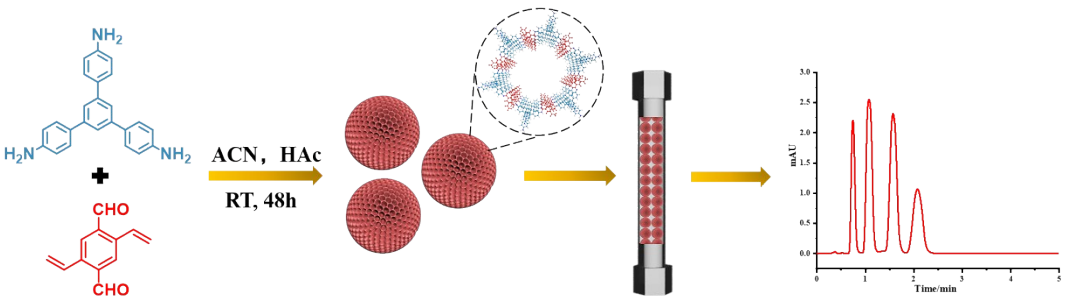

Figure S1. Schematic representation of the synthesis of the S_{COFs} packed column for LC separation.

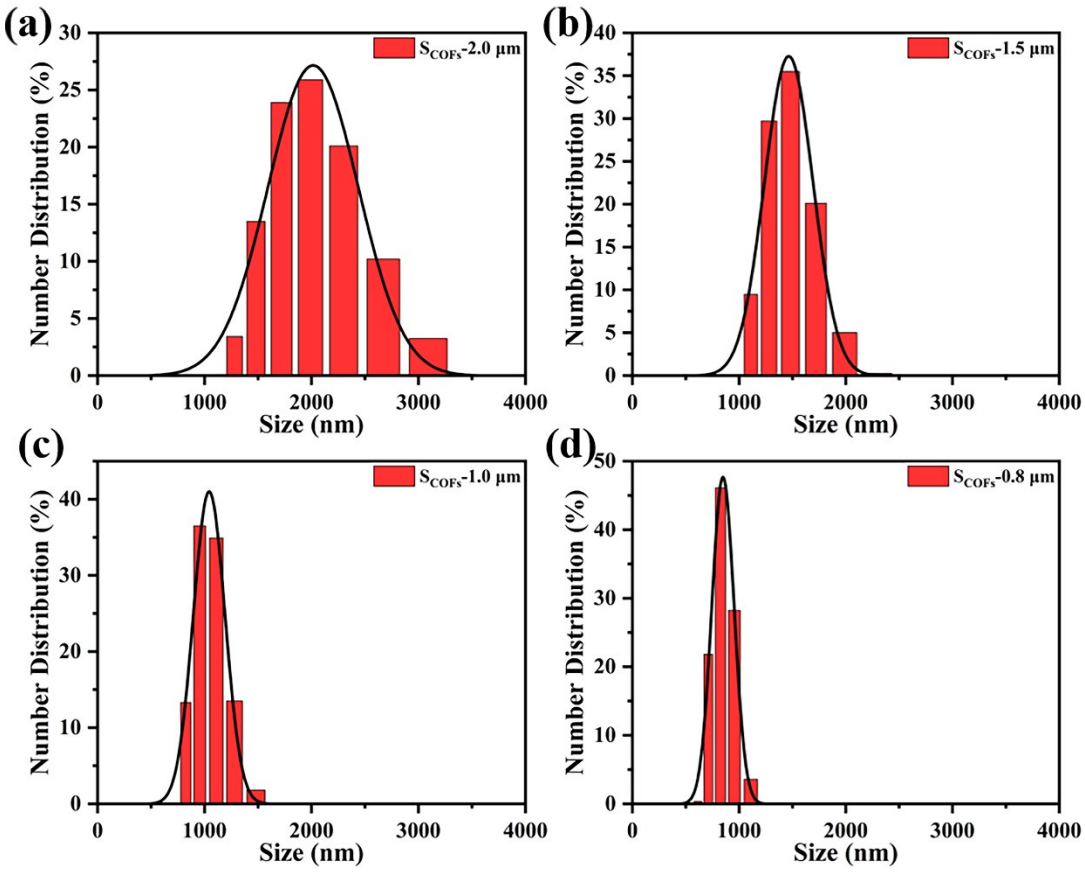

Figure S2. DLS analysis of S_{COFs}-2.0 μm (a), S_{COFs}-1.5 μm (b), S_{COFs}-1.0 μm (c), and S_{COFs}-0.8 μm (d).

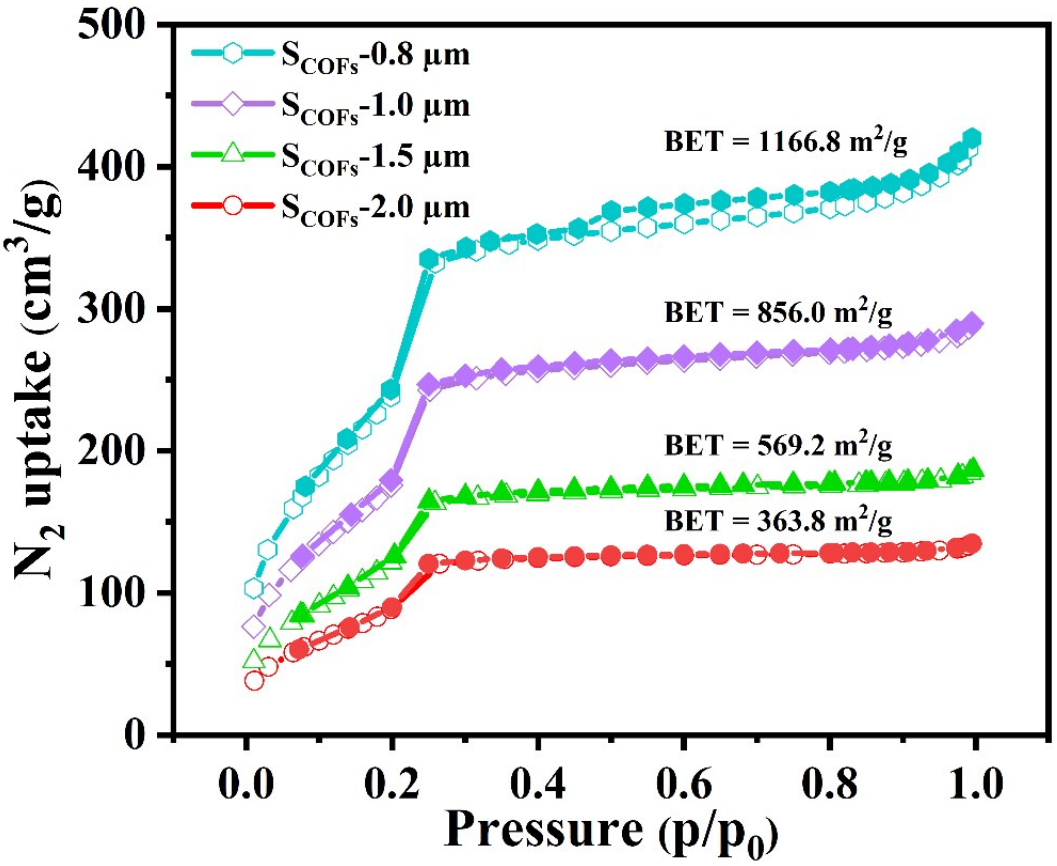

Figure S3. N_2 adsorption−desorption isotherms of S_{COFs}-2.0 μm, S_{COFs}-1.5 μm, S_{COFs}-1.0 μm, and S_{COFs}-0.8 μm.

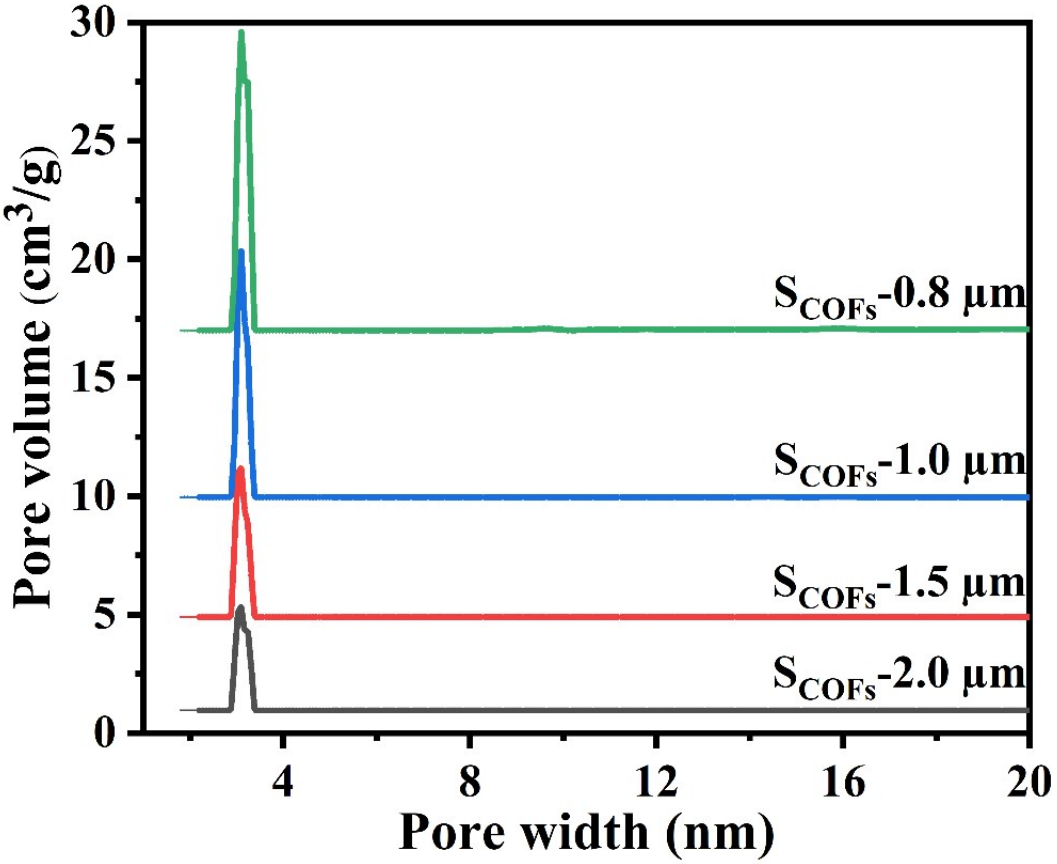

Figure S4. Pore size distribution of the S_{COFs}-2.0 μm, S_{COFs}-1.5 μm, S_{COFs}-1.0 μm, and S_{COFs}-0.8 μm. Pore size distributions of the S_{COFs} were calculated by using the NLDFT model.

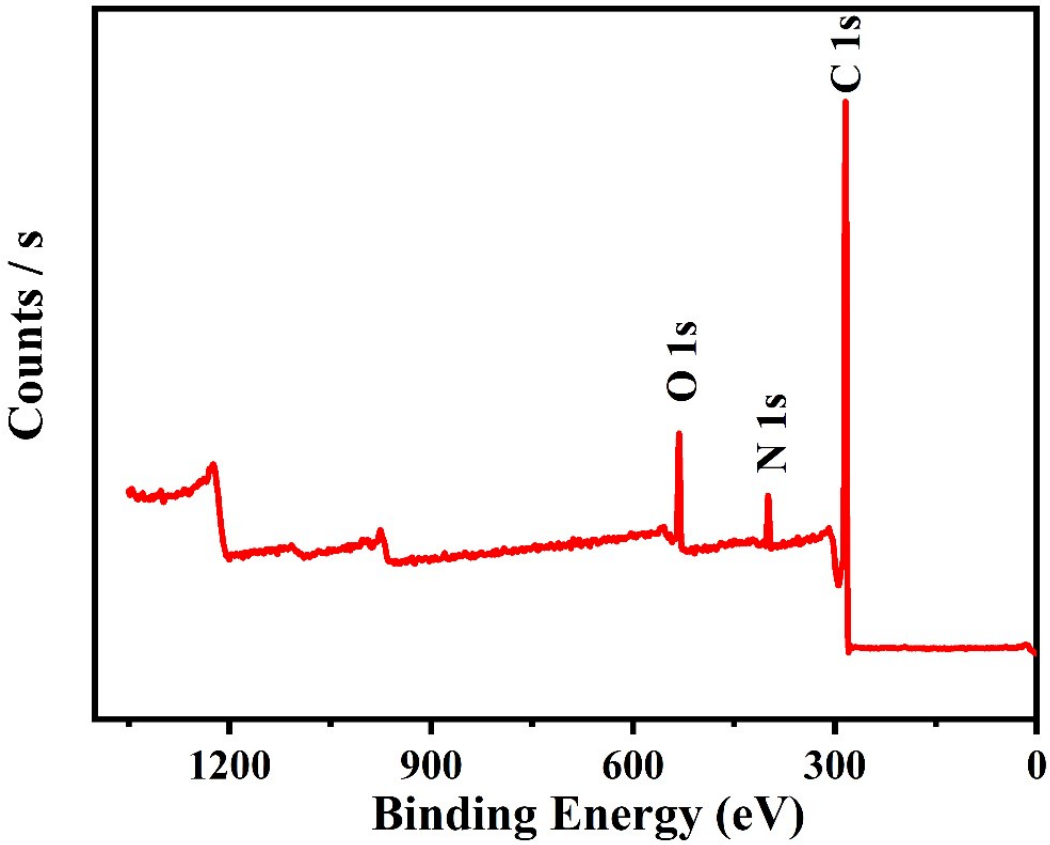

Figure S5. XPS patterns of S_{COFs}-1.0 μm.

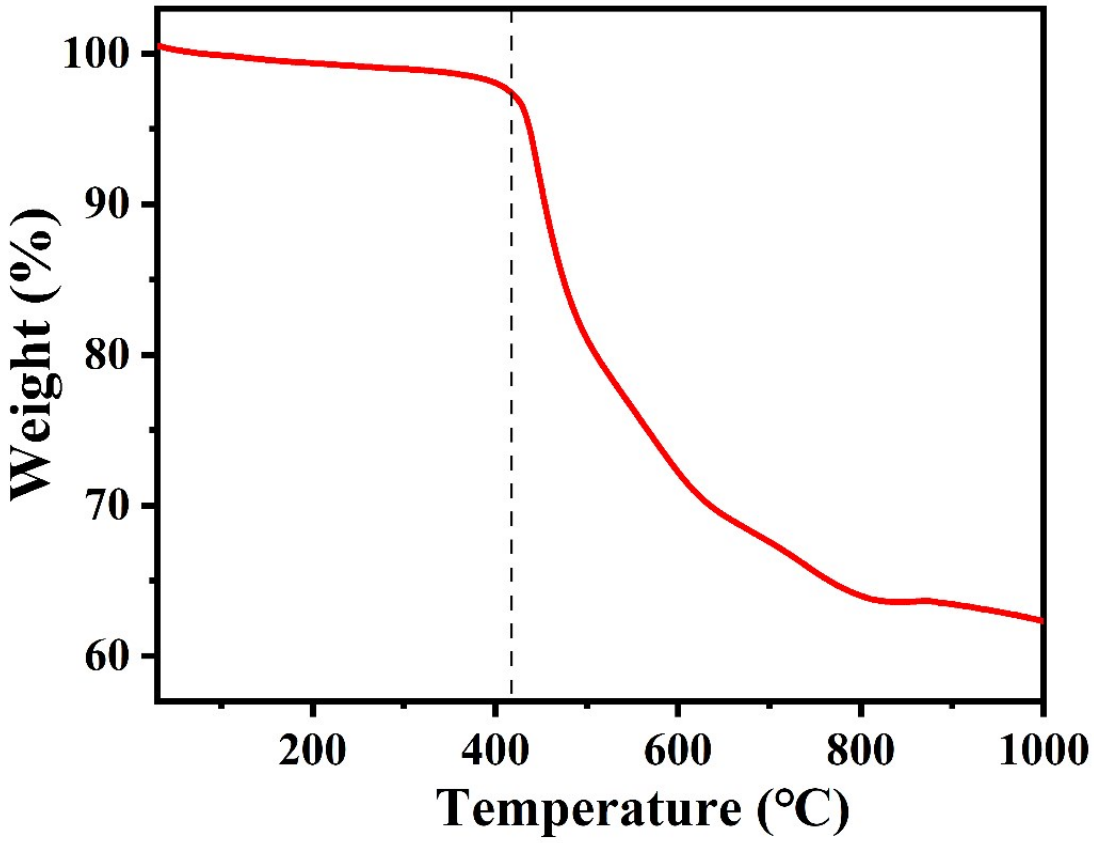

Figure S6. TGA curves of the S_{COFs}-1.0 μm.

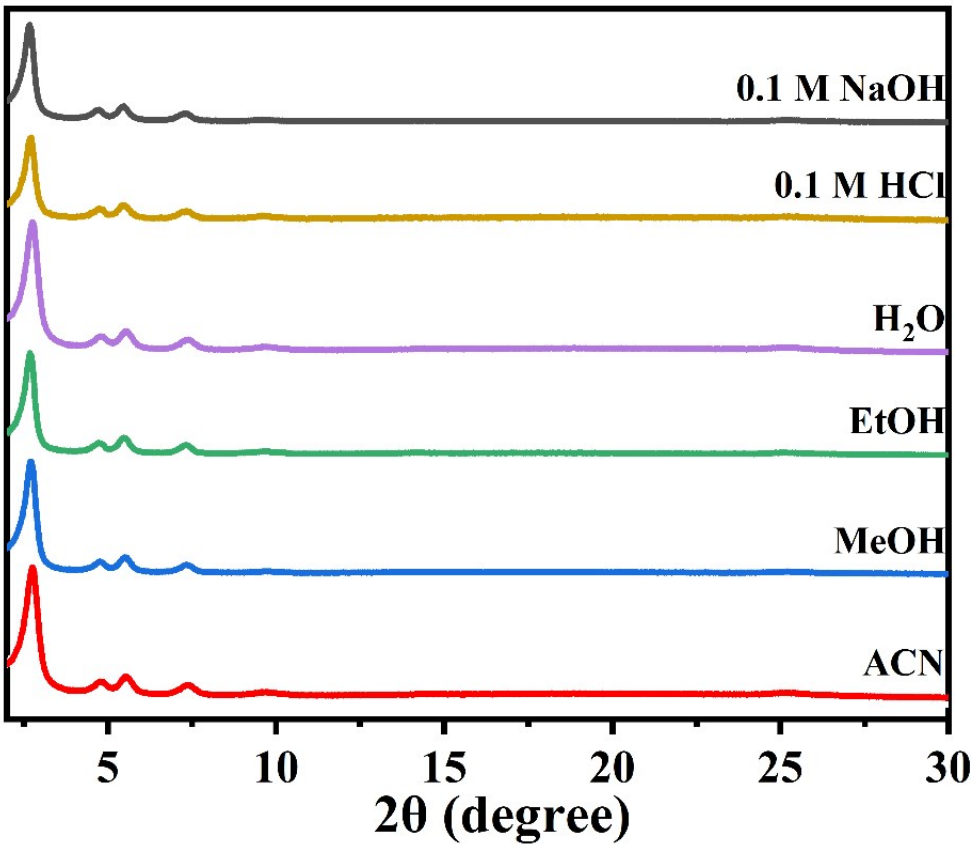

Figure S7. PXRD patterns of the S_{COFs}-1.0 μm after soaking at different solvents for 48 h.

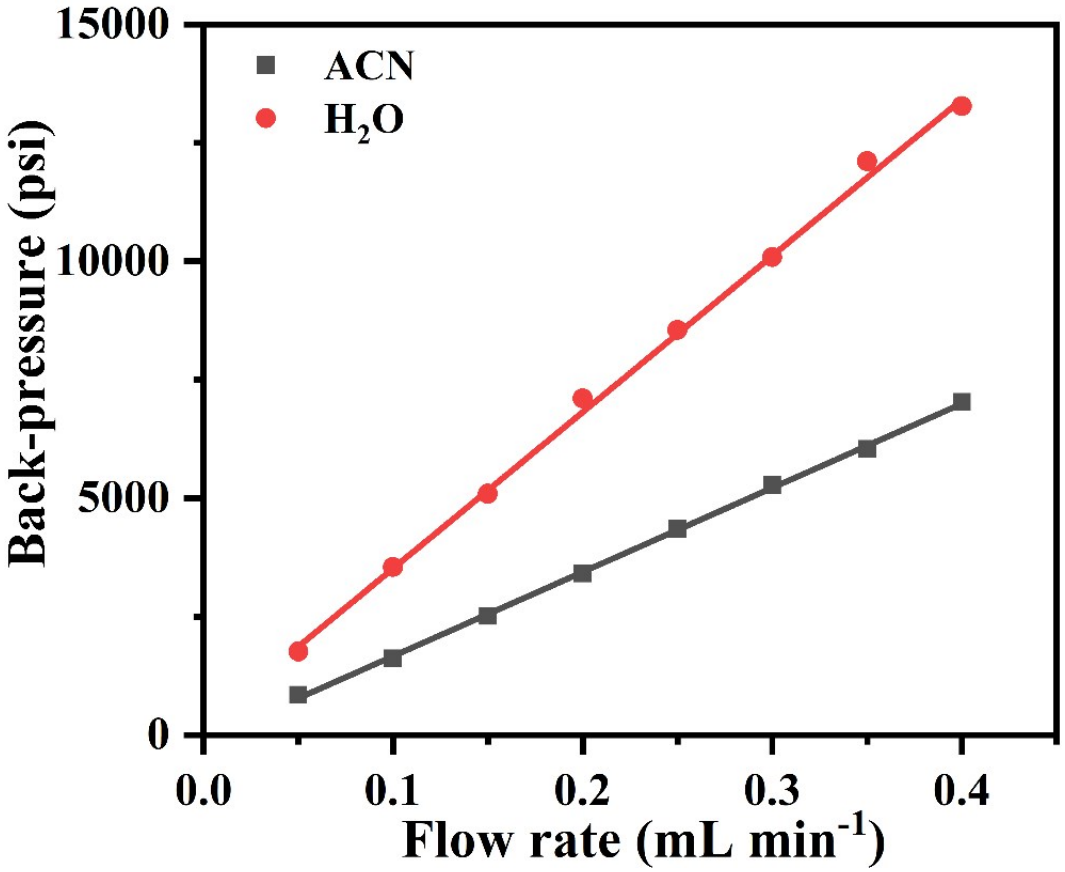

Figure S8. Back pressure against flow rate for S_{COFs}-1.0 μm-packed column. Experimental conditions: mobile phase, pure ACN and pure H_2O; flow rate, 0.05-0.4 mL min^{-1}.

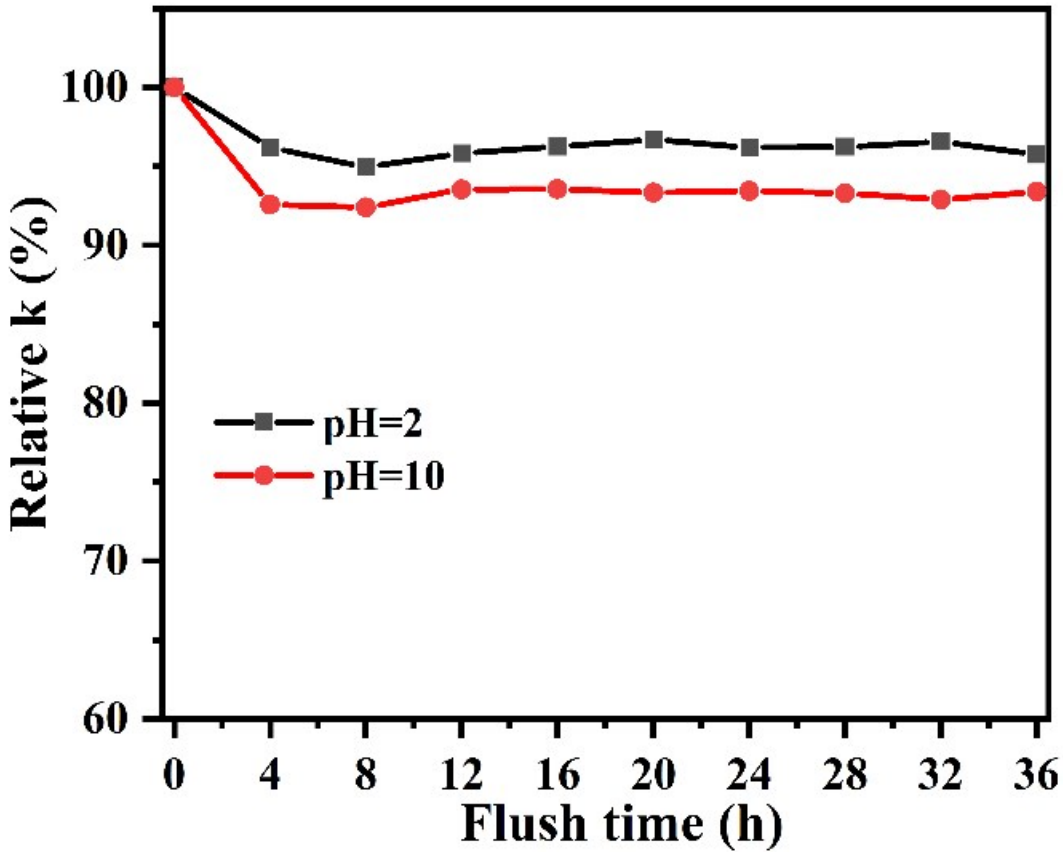

Figure S9. The pH stability of S_{COFs}-1.0 μm-packed column. The column was flushed by 60% ACN with different pH values (pH = 2 and 10). The analytes: toluene. Chromatographic conditions: mobile phase, ACN/H_2O (60/40, v/v); flow rate, 0.2 mL min^{-1}; detection wavelength, 214 nm.

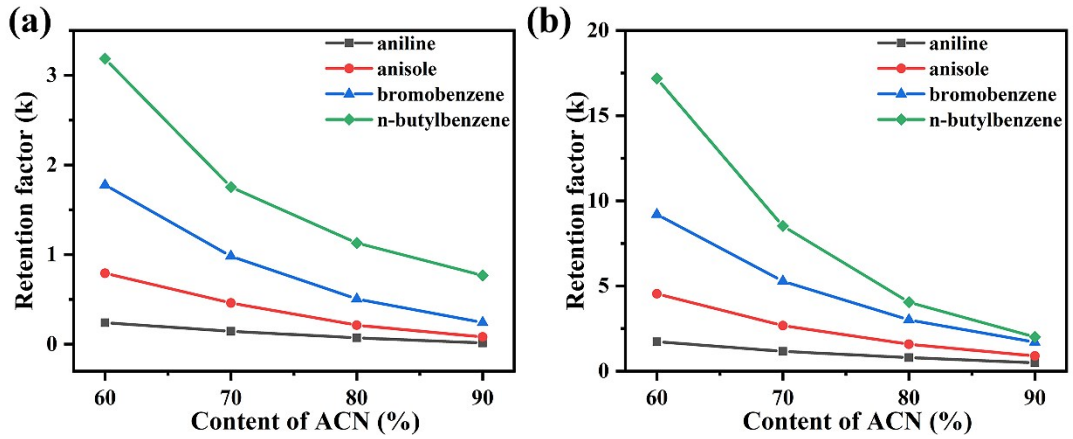

Figure S10. Relationship between retention factor and acetonitrile concentration on the S_{COFs}-1.0 μm-packed column (a) and C18 packed column (b).

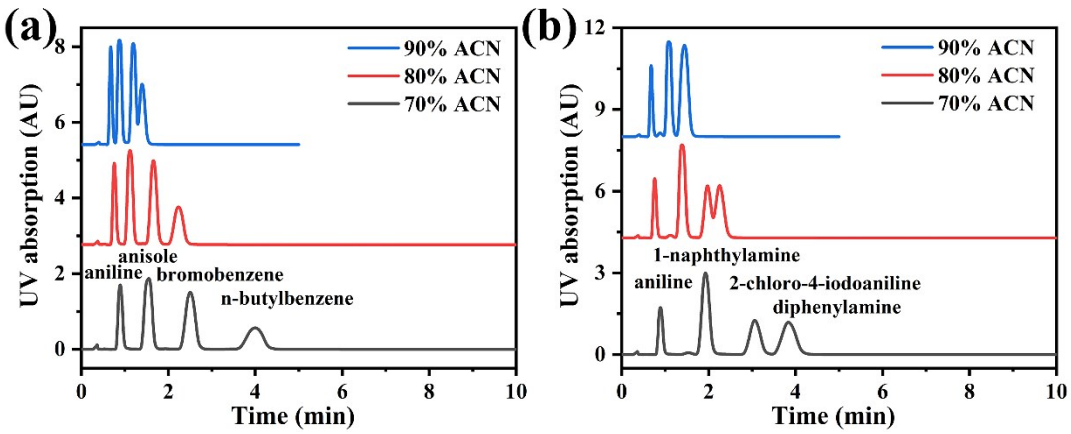

Figure S11. Effect of mobile phase composition on S_{COFs}-1.0 μm-packed column for the separation of monosubstituted aromatics (a) and anilines (b). Conditions: flow rate, 0.2 mL min^{-1}; detection wavelength, 214 nm.

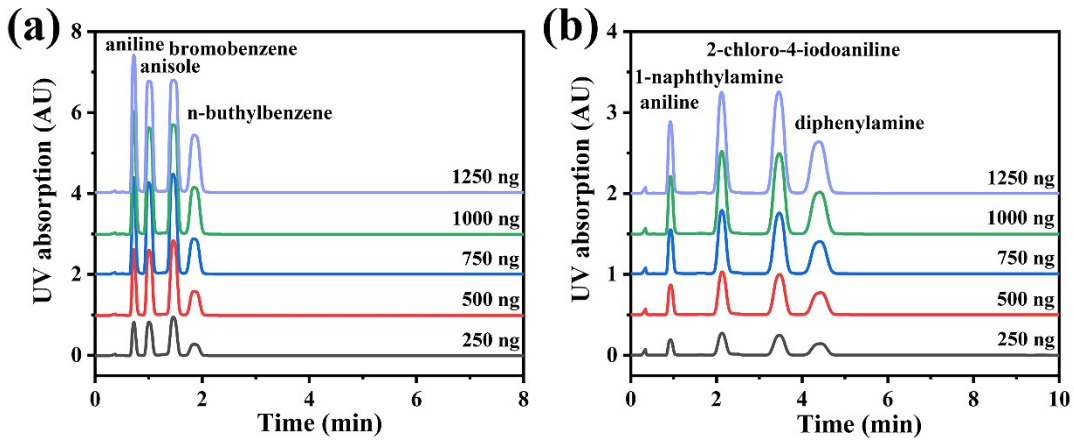

Figure S12. Effect of analyte mass on S_{COFs}-1.0 μm-packed column for the separation of monosubstituted aromatics (a) and anilines (b). Conditions: ACN/H_2O (80/20, v/v) in (a) and ACN/H_2O (70/30, v/v) in (b); flow rate, 0.2 mL min^{-1}; detection wavelength, 214 nm for (a) and 254 nm for (b).

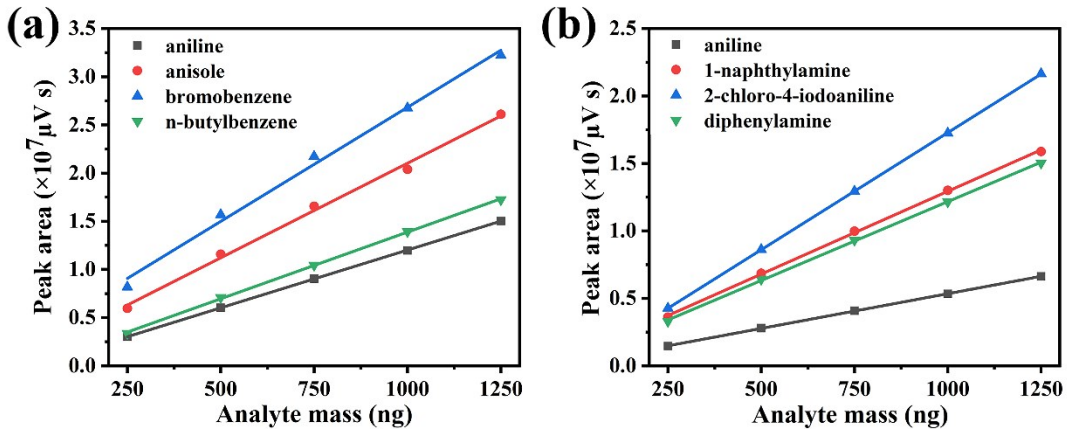

Figure S13. Effect of analyte mass on peak area of monosubstituted aromatics (a) and anilines (b) on S_{COFs}-1.0 μm-packed column.

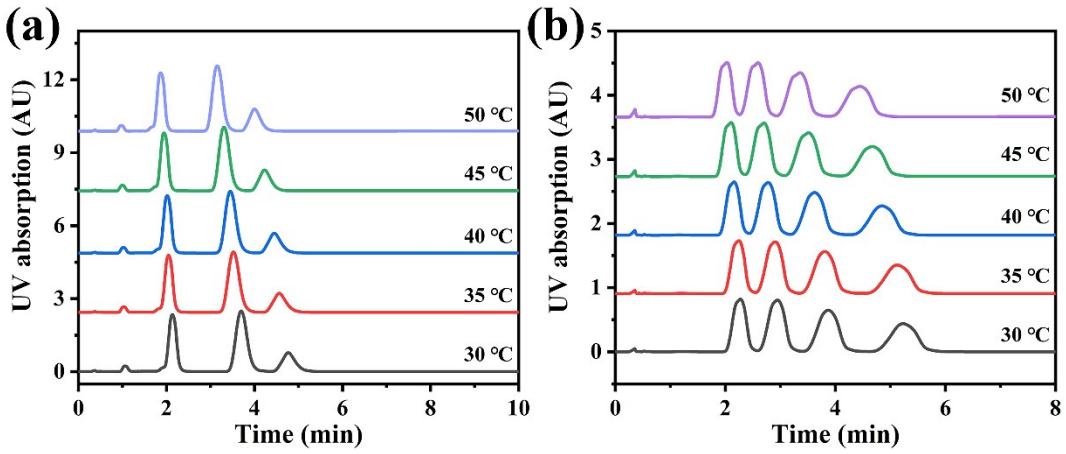

Figure S14. Effect of temperature on S_{COFs}-1.0 μm-packed column for the separation of PAHs (a) and alkylbenzenes (b). Conditions: ACN/H_2O (80/20, v/v) in (a) and ACN/H_2O (65/35, v/v) in (b); flow rate, 0.2 mL min^{-1}; detection wavelength, 214 nm.

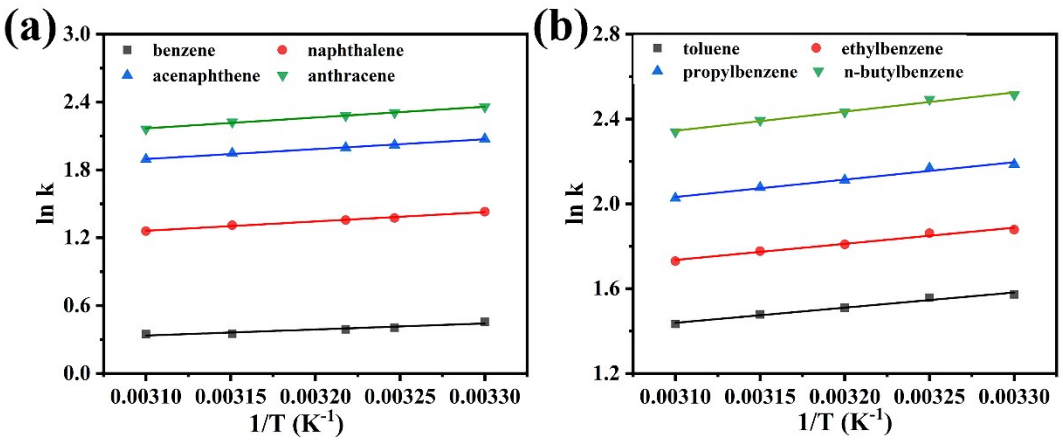

Figure S15. Van't Hoff plots of PAHs (a) and alkylbenzenes (b) on S_{COFs}-1.0 μm-packed column.

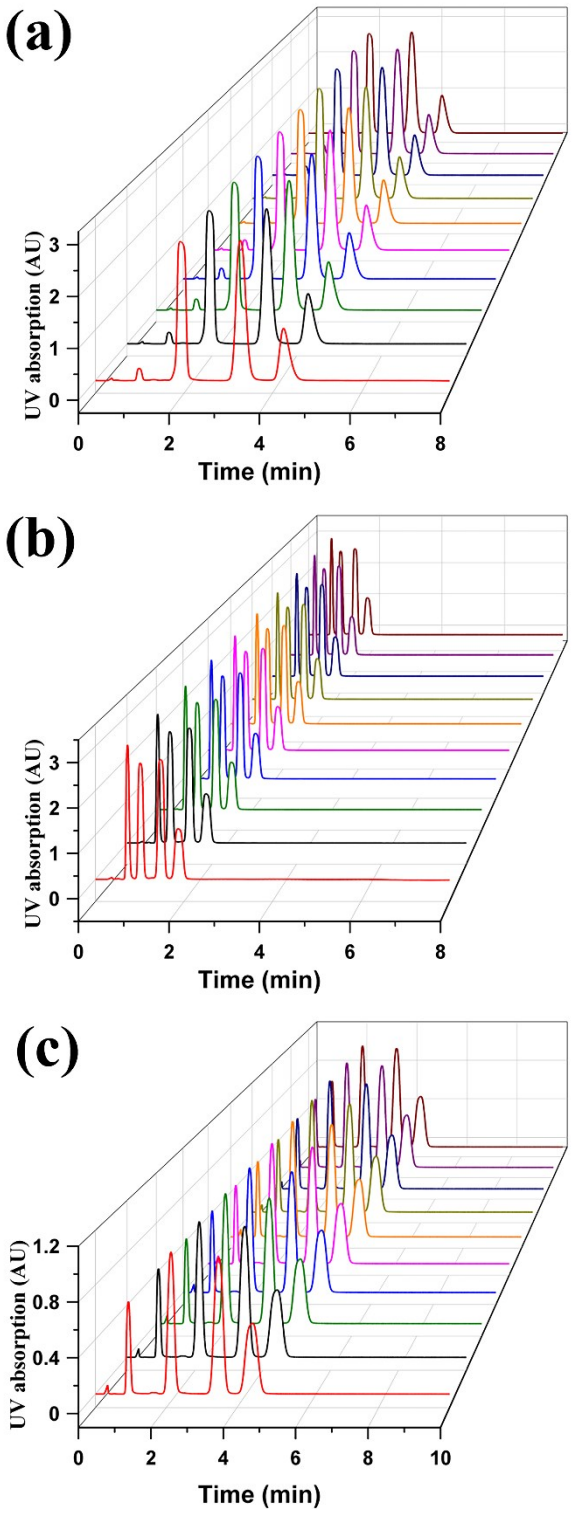

Figure S16. The UPLC chromatograms for continuous 10 times separation of (a) PAHs, (b) monosubstituted aromatics, and (c) anilines on the S_{COFs}-1.0 μm-packed column.

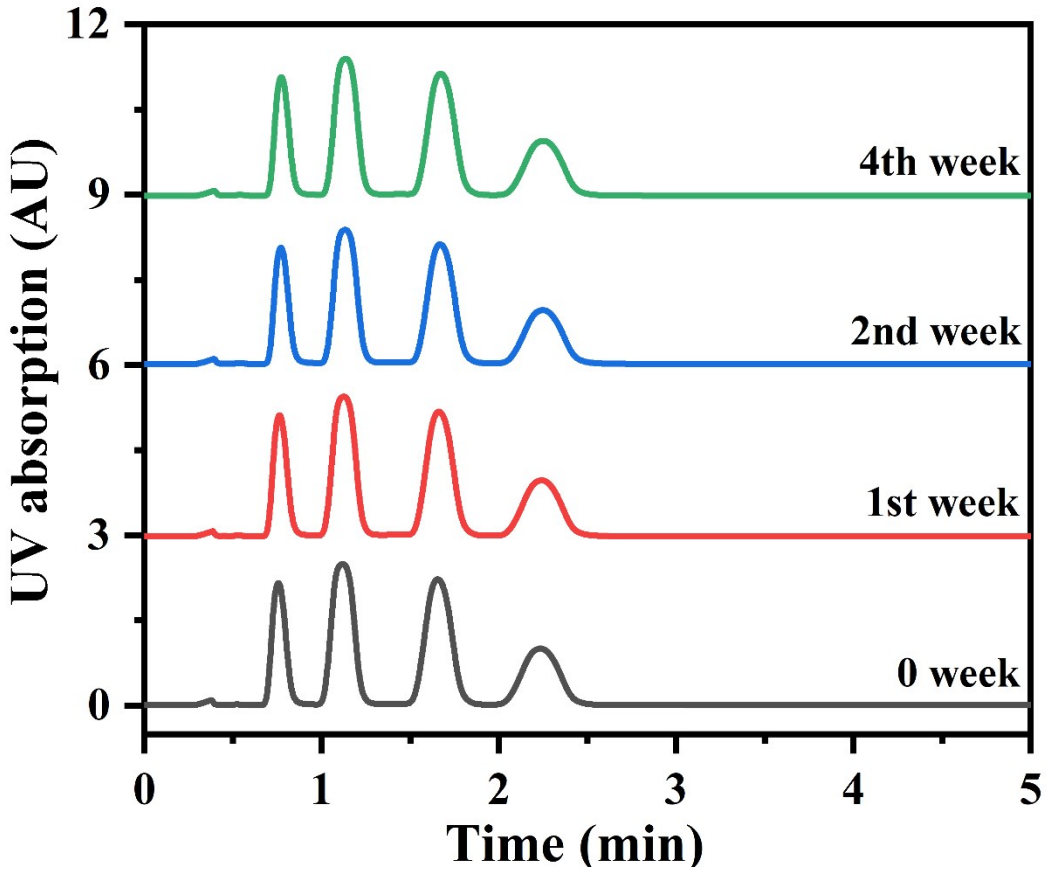

Figure S17. The UPLC chromatograms for separation of monosubstituted aromatics on the S_{COFs}-1.0 μm-packed column at different times.

Table S1. The composition of each element in S_{COFs}-1.0 μm calculated from the XPS analysis.

	Elements (%)		
	C	N	O
S_{COFs}-1.0 μm	86.32	5.28	8.40

Table S2. Vandeemter Coefficients obtained from the fitting curve of Figure 2b.

Analyte	S_{COFs}-1.5 µm			S_{COFs}-1.0 µm		
	A/µm	B/µm^{2}s^{-1}	C/ms	A/µm	B/µm^{2}s^{-1}	C/ms
anthracene	**22.77**	**47226**	**64.42**	**11.61**	**6232**	**2.52**

Table S3. The log K_{ow} values of the studied analytes.

Analytes	Log K_{ow} [a]	Analytes	Log K_{ow} [a]
aniline	0.90	ethylbenzene	3.15
1-naphthylamine	2.25	propylbenzene	3.69
2-chloro-4-iodoaniline	2.89	n-butylbenzene	4.38
diphenylamine	3.50	o-nitrofluorobenzene	2.01
anisole	2.11	o-chloronitrobenzene	2.46
bromobenzene	2.99	1-bromo-2-nitrobenzene	2.70
benzene	2.13	1-iodo-2-nitrobenzene	2.98
naphthalene	3.30	dimethyl phthalate	1.60
acenaphthene	3.92	dipropyl phthalate	3.27
anthracene	4.45	dibutyl phthalate	4.50
toluene	2.73	dicyclohexyl phthalate	6.20

[a]: Data from: https://pubchem.ncbi.nlm.nih.gov/ and http://www.chemspider.com/

Table S4. The resolution value of PAHs on C18 packed column and S_{COFs}-1.0 μm-packed column.

	C18	S_{COFs}-1.0 μm
benzene/naphthalene	2.146	2.814
naphthalene/acenaphthene	2.180	2.596
acenaphthene/anthracene	1.245	1.504

Table S5. Thermodynamic parameters for short-column liquid chromatographic separation of the studied analytes on the S_{COFs}-1.0 μm-packed column. (T = 303.15 K)

Analytes	ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (KJ mol^{-1})	R^2
benzene	-4.45 ± 0.37	11.34 ± 1.19	-7.89 ± 0.73	0.9802
naphthalene	-6.87 ± 0.34	10.81 ± 1.11	-10.15 ± 0.67	0.9925
acenaphthene	-7.27 ± 0.30	6.77 ± 0.96	-9.32 ± 0.59	0.9950
anthracene	-7.95 ± 0.28	6.64 ± 0.90	-9.96 ± 0.55	0.9963
toluene	-5.95 ± 0.50	6.47 ± 1.59	-7.91 ± 0.97	0.9795
ethylbenzene	-6.33 ± 0.52	5.20 ± 1.66	-7.91 ± 1.01	0.9802
propylbenzene	-6.82 ± 0.54	4.23 ± 1.74	-8.10 ± 1.06	0.9813
n-butylbenzene	-7.50 ± 0.54	3.76 ± 1.73	-8.64 ± 1.06	0.9847

Table S6. Precision for analytes on the S_{COFs} -1.0 μm-packed column.

Analytes	RSD (%) (n=10)		
	T	Peak height	Peak area
benzene	0.169	0.698	0.595
naphthalene	0.605	0.229	0.510
acenaphthene	0.610	0.139	0.259
anthracene	0.056	0.124	0.556
aniline	0.320	0.057	0.157
anisole	0.478	0.104	0.110
bromobenzene	0.388	0.270	0.089
butylbenzene	0.386	0.601	0.929
naphthylamine	0.887	0.335	0.317
2-chloro-4-iodoaniline	0.156	0.0502	0.201
diphenylamine	0.197	0.047	0.178