Supporting Information

A MOF with high specific surface area for rapid separation and determination of β-adrenergic receptor blockers in pork by open-tubular capillary electrochromatography

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1. Experimental Methods

1.1 Preparation of NH_2-MIL-125

The preparation of the MOF material NH_2-MIL-125 was based on the previous research literature[1], a solvothermal method was employed with the detailed experimental procedure described as follows. First, 2-aminoterephthalic acid (0.816 g) was accurately weighed and dissolved in a mixed solution of N,N-dimethylformamide (DMF) and methanol (MeOH), and the mixture was gently stirred to ensure complete dissolution of 2-aminoterephthalic acid. Subsequently, titanium(IV) isopropoxide (0.45 mL) was added to the above homogeneous solution. After the addition was completed, the reaction mixture was subjected to continuous magnetic stirring at room temperature for 30 min to achieve sufficient mixing of the reactants. The resulting solution was transferred into a 50 mL Teflon-lined stainless steel autoclave and heated in an oven at 150 °C for 24 h. After the autoclave was naturally cooled to room temperature, the product was washed twice with DMF and anhydrous MeOH, followed by centrifugation at 8000 rpm for 5 min to remove unreacted impurities. Finally, the collected solid product was dried overnight in a vacuum oven at 60 °C to eliminate residual solvents adsorbed in the pores of the MOF. The synthesis route of NH_2-MIL-125 was illustrated in Fig. S1.

1.2 Preparation of aldehyde-treated column

The aldehyde-treated column was obtained by the following three steps:

(1) Pretreatment of capillary column: The bare capillary column was continuously washed with 1 mol/L of HCl for 1 h. Then the capillary column was flushed with ultra-pure water until the solution in the column was neutral. Finally, the capillary column was continuously washed with 1 mol/L of NaOH for 30 min. The obtained capillary column with sealing both ends was placed in a water bath at 40 ℃ for 12 h. After that, the column was rinsed with ultra-pure water to neutral, and then rinsed with methanol for 15 min to wash away the residue, and dried with nitrogen stream.

(2) Aminated column: The pretreated capillary column was flushed with the solution of APTES in methanol (V/V=1/1) for 8 min, then the capillary column with sealing both ends was placed in a water bath at 40℃ for 24 h. Aminated column was

obtained.

(3) Aldehyde-treated column: 50 % glutaraldehyde solution was diluted 25 times with ultra-pure water to prepare 2 % glutaraldehyde solution, and then the pH value of the solution was adjusted to 11 by adding drop by drop 1.0 mol/L NaOH solution. Finally, the prepared solution was injected into the aminated column for 1 h. Aldehyde-treated was obtained.

1.3 Preparation and composition of ammonium acetate buffers

The ammonium acetate buffers were prepared by titrating ammonium acetate, ammonia and acetic acid at an equal molar concentration, or by adjusting with ammonia and acetic acid until the desired pH value was reached.

1.4 Preparation of sample solutions

Each of the three β-adrenergic receptor blockers (10.0 mg) was accurately weighed, then dissolved in an according ammonium acetate buffer (10 mL) to prepare a mixed sample solution with each component at 1.0 $mg \cdot mL^{-1}$. Refrigerated for use.

Sample solutions (1.0 $mg \cdot mL^{-1}$) of β-adrenergic receptor agonists and sulfonamide antibiotics were prepared according to the same method mentioned above.

1.5 CEC calculation formulas

In the CEC analysis results, the resolutions (Rs) of all analytes were calculated according to Equations (1) :

$$Rs=1.18(t_2-t_1)/(W_{1/2(1)}+W_{1/2(2)}) \quad (1)$$

Here, t1, $W_{1/2(1)}$ and t_2 $W_{1/2(2)}$ represented the retention time (min) and the peak width at half height (min) of each analyte.

In the CEC analysis results, the Number of theoretical plates (N) of all analytes were calculated according to Equations (2):

$$N = 5.54\ (t_R/W_{1/2})^2 \quad (2)$$

Here, t_R is the retention time (min), and $W_{1/2}$ is the peak width at half height (min).

Electroosmotic flow ($\mu_{EOF,}$ $cm^2V^{-1}S^{-1}$) was determined with thiourea as a neutral marker and calculated according to Equation (3):

$$\mu_{EOF}=L\times l/(V\times t_0) \quad (3)$$

Here, L and l represented the total length (cm) and the effective length (cm) of the OT column; V was separation voltage (kV) and t_0 was the retention time of thiourea (s).

1.6 Detection of β-adrenergic receptor blockers in real pork samples

Certain unscrupulous livestock farmers may illegally administer β-adrenergic receptor blockers (e.g., Prop). These substances inhibit fat deposition in pigs while promoting muscle growth, thereby increasing the market value of pork. However, when humans ingest these residues through pork consumption, β-adrenergic receptor blockers can accumulate in the body and exert unintended pharmacological effects, posing potential health hazards. Therefore, developing a rapid and simple detection method for β-adrenergic receptor blockers in pork is of great significance.

In this study, a method for the separation and detection of β-adrenergic receptor blockers was established using an NH_2-MIL-125 bonded OT column. Pork was used as the analytical sample, and the target analytes were successfully detected in real pork samples.

(1) Linear range and detection limit: A series of standard solutions of β-adrenergic receptor blockers (0.01-1.00 mg/mL) were prepared and filtration through a 0.45 μm membrane. Separation and detection were then performed using a CEC system under optimized conditions. Linear curves were constructed by plotting the peak areas (y) of each analyte against its corresponding standard concentration (x, mg/mL), from which linear equations and correlation coefficients (R^2) were obtained. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on signal-to-noise (S/N) ratio of 3 and 10, respectively.

(2) Precision: A 0.10 mg/mL standard solution of β-adrenergic receptor blockers was prepared and analyzed in seven replicates under the optimized CEC conditions. The RSDs for retention time and peak area were calculated.

(3) Accuracy: To further validate the practical applicability of the NH_2-MIL-125 bonded OT column, pork real samples were selected. Pork samples were pretreated according to the method in the import-export industry standard (SN/T 5593-2024)

with a slight modification: the reconstitution solution was replaced with methanol. The pretreated samples were analyzed using the standard method specified in SN/T 3235-2012, and recovery experiments were performed via the spiking method. A series of standard solutions of the three β-adrenergic receptor blockers ranged from 0.01 mg/mL to 1.00 mg/mL were determined under the optimal CEC conditions for plotting the calibration curves and calculating the regression equations. According to the calibration curves, the concentrations of spiked samples were collected as 0.01 mg/mL, 0.10 mg/mL, and 0.50 mg/mL, respectively. Seven parallel experiments were conducted to confirm the accuracy of the method. In this section, all analytical solutions were filtered through a 0.22 μm membrane prior to detection under optimal CEC conditions.

(4) Three β-adrenergic receptor blockers detemination in real pork sample: The sample solution obtained in 1.6(3) was analyzed under the optimal CEC conditions.

2. Supporting Figures

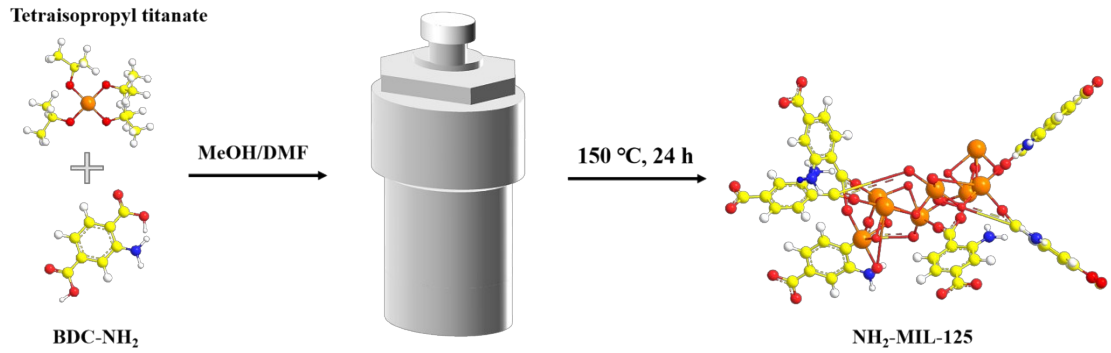


Fig. S1 Schematic of the synthesis of NH_2-MIL-125

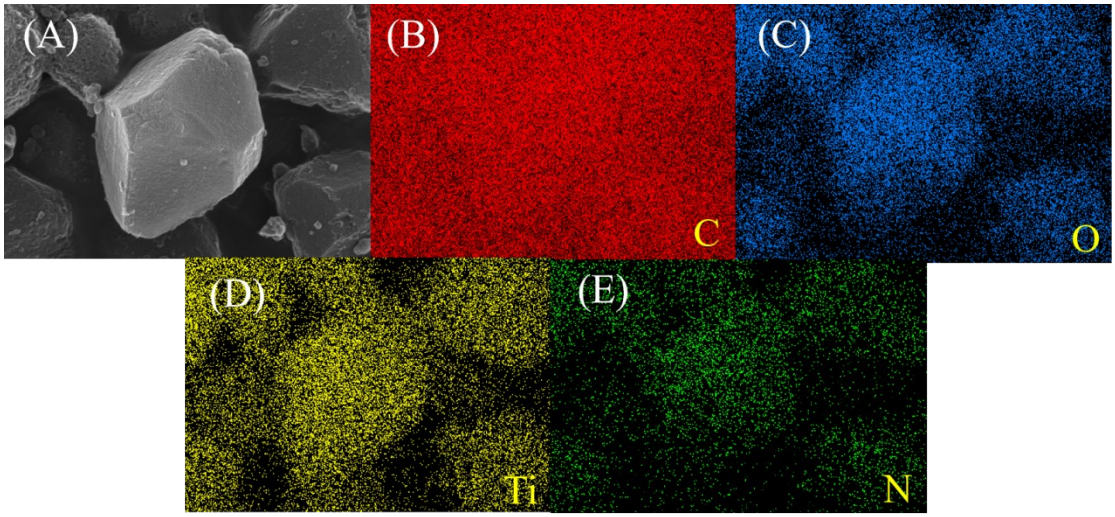


Fig. S2 SEM images (A) and EDS mapping (B-E) of NH_2-MIL-125

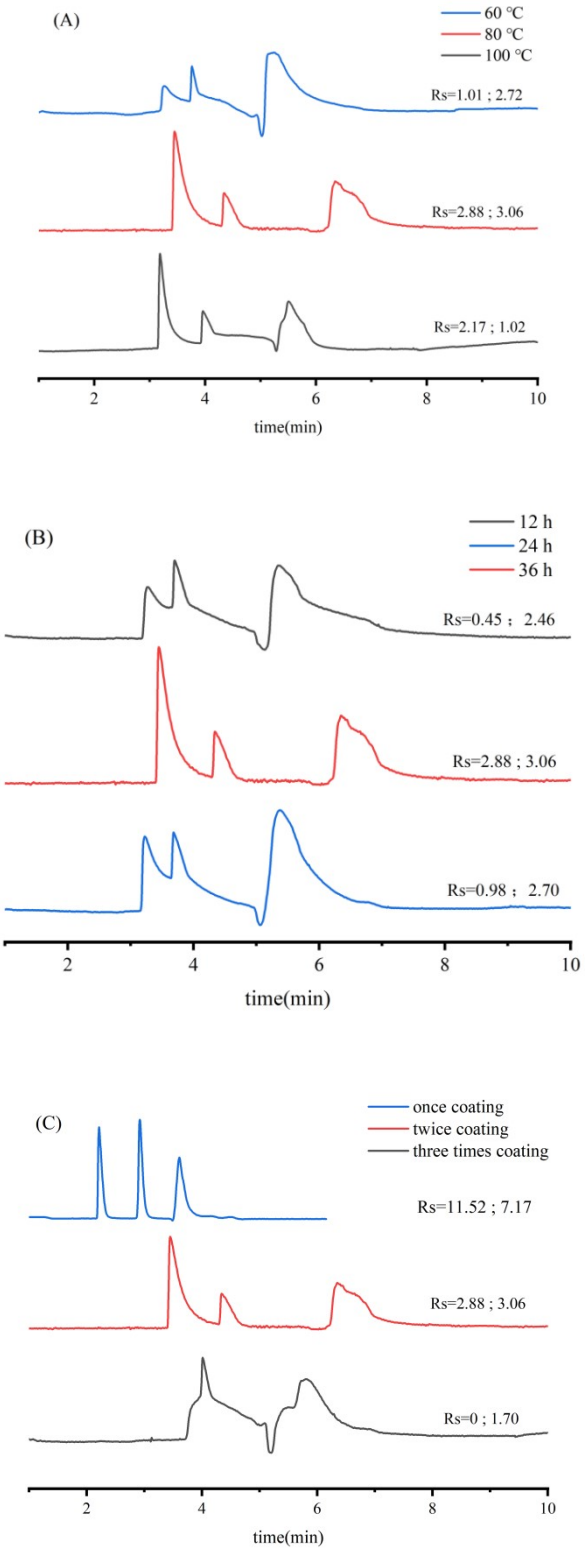


Fig. S3 Optimization of column preparation conditions: temperature (A), time (B) and coating time (C). (Other preparation conditions: 24h, twice coating (A); 80 ℃, twice coating (B); 80 ℃, 24h (C); CEC conditons: sample, 1.0 mg/mL; 20 mmol/L acetate buffer, pH=10; voltage, 15 kV)

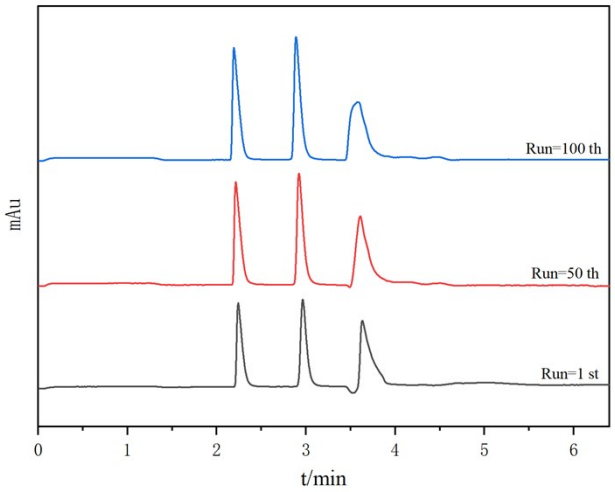


Fig. S4 Chromatograms of three β-adrenergic receptor agonists at different number of runs

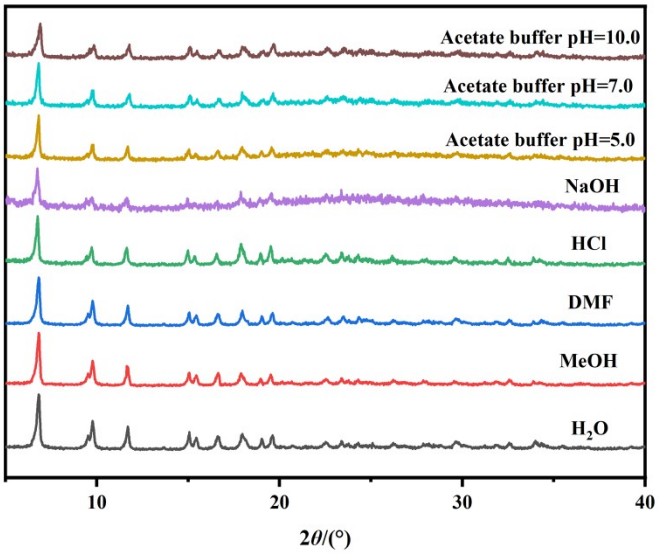


Fig. S5 XRD patterns of NH_2-MIL-125 after soaking in different buffers and solvents

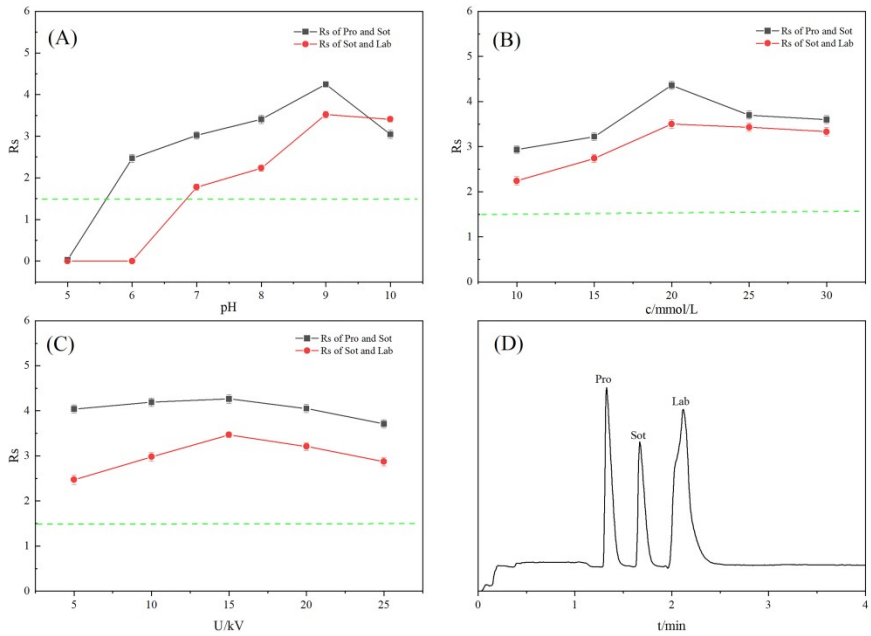


Fig. S6 Effect of buffer pH (A) buffer concentration (B) and operation voltage (C) on the *Rs* of β-adrenergic receptor blockers. (Experimental conditions: 1.0 mg/mL, detection wavelength 250 nm, 20 mmol/L acetate buffer, voltage 15 kV (A), pH=9, voltage 15 kV (B), 20 mmol/L acetate buffer, pH=9 (C))

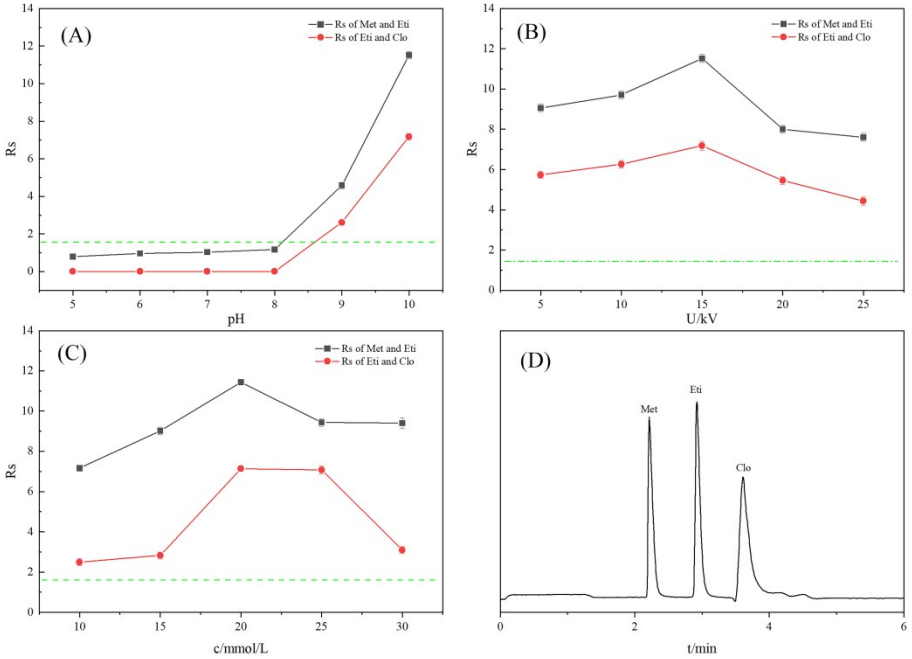


Fig. S7 Effect of buffer pH (A) buffer concentration (B) and operation voltage (C) on the *Rs* of β-adrenergic receptor agonists. (Experimental conditions: 1.0 mg/mL, detection wavelength 250 nm, 20 mmol/L acetate buffer, voltage 15 kV (A), pH=10, voltage 15 kV (B), 20 mmol/L acetate buffer, pH=10 (C))

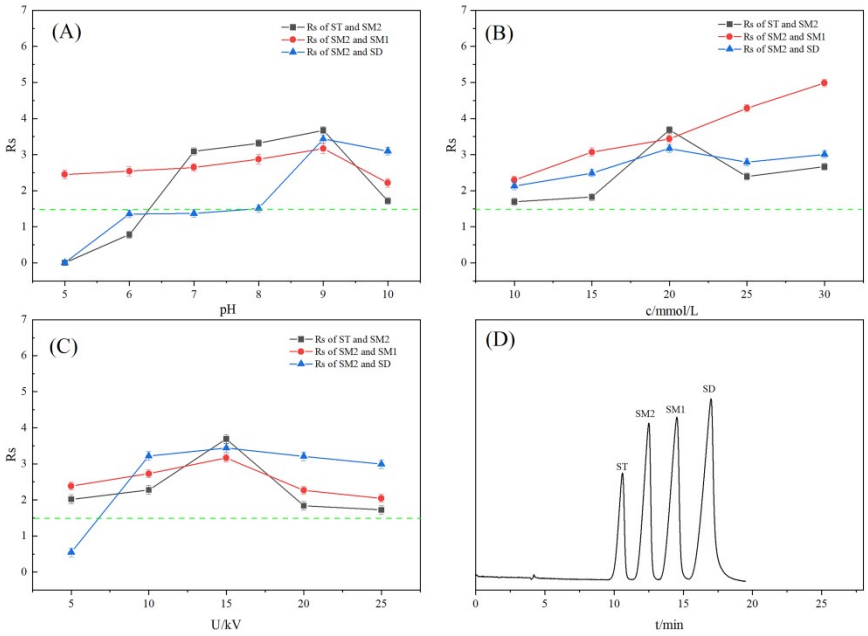


Fig. S8 Effect of buffer pH (A) buffer concentration (B) and operation voltage (C) on the *Rs* of sulfonamides (Experimental conditions: 1.0 mg/mL, detection wavelength 250 nm, 20mmol/L acetate buffer, voltage 15 kV (A), pH=9, voltage 15 kV (B), 20 mmol/L acetate buffer, pH=9 (C))

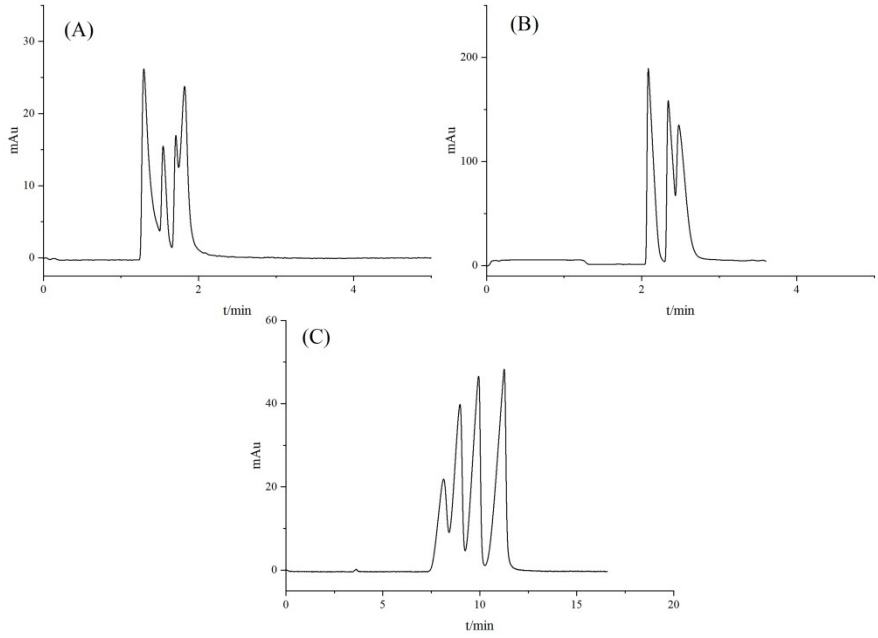


Fig. S9 Separation chromatograms of three β-adrenergic receptor agonists (A), three β-adrenergic receptor blockers (B) and four sulfonamide antibiotics (C) by the bare column. (Experimental conditions: pH = 8.0, 10.0, 8.0, respectively; 10 mmol/L acetate buffer; voltage, 15 kV)

3. Supporting Tables:

Table S1 EDS data of NH_2-MIL-125

Elements	Atomic%
C	71.42
O	3.28
N	19.08
Ti	6.22

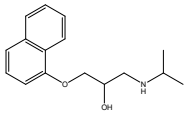
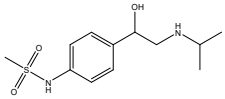
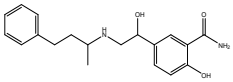
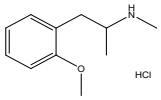
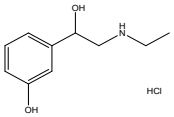
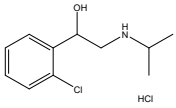
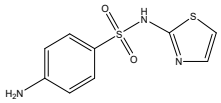
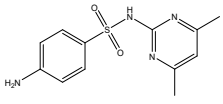
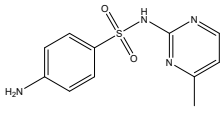
Table S2 Pore structure parameters of NH_2-MIL-125

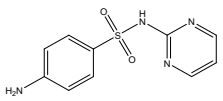
S_{BET} (m^2/g)	Pore volume (mL/g)	Pore size (nm)
1247.57	0.61	1.76

Table S3 Repeatability and stability of the NH_2-MIL-125 bonded OT column

Types and numbers (n)	RSDs (%) of retention time			RSDs (%) of column efficiency		
	Meth	Eti	Clo	Meth	Eti	Clo
Run to run (n=7)	2.54	1.55	1.33	1.89	1.67	2.01
Day to day (n=7)	3.02	1.22	1.90	3.01	2.98	2.30
Column to column (n=3)	3.60	2.80	2.92	2.92	3.04	3.44
Runs (n=100)	2.97	2.76	2.55	3.27	4.12	4.51

Table S4 Separation results of NH_2-MIL-125 bonded OT column and basic properties of the analytes

	Analytes	Structure	Molecular volume (Å^3)	Molecular weight (g/mol)	LogP*	pKa*	Optimal pH	Analysis time# (min)	*Rs*	N (plates/m)
β-Adrenergic blockers	Prop		12.6×7.2×3.4	259.34	3.4	13.84	9	1.33	-	6950
	Sot		10.8×6.5×3.1	272.36	0.1	8.20		1.67	4.37	10973
	Lab		13.5×7.8×3.6	328.41	1.8	7.41		2.13	3.50	3510
β-adrenergic agonists	Meth		11.8×6.9×3.2	215.72	1.5	8.6-8.8	10	2.24	-	27732
	Eti		10.5×6.3×3.0	223.71	0.5	8.9-9.1		2.97	11.52	44601
	Clo		12.2×7.1×3.3	250.17	1.0	8.5-8.7		3.63	7.17	18748
Sulfonamide antibiotics	ST		11.0×7.0×3.5	255.32	0.9	7.2	9	10.60	-	12809
	SM2		12.0×7.0×3.5	278.33	1.3	7.4/2.65		12.52	3.69	12149
	SM1		11.7×7.0×3.4	264.31	1.0	2.29		14.55	3.17	10575

SD		10.9×6.7 ×3.2	250.28	0.8	2.21		17.03	3.44	13519

*pKa and logP values were from https://baike.baidu.com/ and/or https://www.chemicalbook. com/.

#represents the shortest analytical time

1 Ma Ruiying, Ma Di, Zhao Xiaoxia, et al. Preparation and Photocatalytic Performance of Different Crystal Morphologies NH_2-MIL-125(Ti) [J]. ***Fine Chemicals***, 2019, 36(03): 481-486. DOI: 10.13550/j.jxhg.20180555.