Supporting Information

Separation of dithiothreitol and dithioerythritol by an open tubular capillary electrochromatographic column with a MOF modified by histidine as stationary phase

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1. Experimental Methods:

1.1 Preparation of ZIF-93

ZIF-93 was prepared according to the previous reported method[1]. 367 mg of $Zn(CH_3COO)_2 \cdot 2H_2O$ and 550 mg of aImeIm were accurately weighed and added to 25 mL of methanol. After the mixture ultrasonically dissolving, it was transferred into a 50 mL Teflon lined autoclave and sealed. The autoclave was held at 85 ℃ for 24 h. After the reaction, reaction mixture was centrifuged. The precipitate was washed with methanol three times, then dried under vacuum at 85 ℃ overnight.

1.2 Preparation and composition of borax-HCl buffer

Weigh 38.14 g of borax ($Na_2B_4O_7 \cdot 10H_2O$) and dissolve in 1.0 L of ultrapure water to obtain the stock borax solution of 100 mM.

The 10-40 mM Borax-HCl buffer with pH 7.0-9.0 were prepared by first diluting the stock borax solution the desired concentration, and then by titration of the borax solutions with HCl to the desired pH value.

1.3 Preparation of the sample solution

DTT and DTE powder was kept in -20 °C freezer before use. The standard solution (10.0 mg/mL) of DTT and DTE was made by dissolving DTT and DTE in a small volume of ultrapure water individually, then being diluted with ultrapure water, and stored at 4 °C in a refrigerator (Just in time). The test solutions were obtained by mixing the standard solutions or diluting the mixture standard solutions to appropriate concentrations. Before use, all the solutions were filtered through a 0.45 μm membrane and degrassed by sonication.

1.4 CEC calculation formulas

In the CEC analysis results, the resolutions (Rs) and the selectivity factors (α) between DTE and DTT are calculated according to Equations (1) and (2):

$$Rs=2(t_2-t_1)/(W_1+W_2) \quad (1)$$

$$\alpha=t_2/t_1 \quad (2)$$

Here, t_1, W_1 and t_2 W_2 represent the retention time and the peak widths of DTE and DTT

(s), respectively.

Electroosmotic flow (μ_{EOF}) is 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 represent the total length (cm) and effective length (cm) of the capillary column; V is the CEC separation voltage (kV) and t_0 is the retention time of thiourea (s).

Column efficiency are calculated according to Equation (4):

$$N=5.54(t_R/W_{1/2})^2/l\times 100 \quad (4)$$

Here, t_R and $W_{1/2}$ represent the retention time and the half-peak width of DTE or DTT (s), respectively; l represents the effective column length (cm).

1.5 CEC conditions

All CEC experiments were conducted on an Agilent 7100 CE system (Waldbronn, Germany) equipped with a diode array detector, and operated at room temperature. The capillary column of 75 μm i.d. and 375 μm o.d. from Yongnian Optic Fiber Factory (Handan, China) was used. Total length and the effective length of the prepared His-ZIF-93 column were 34.0 cm and 25.5 cm, respectively. Borax-HCl buffer was used (SI 1.2). Pressure injection was 50 mbar × 3 s. The detection wavelength of DTT and DTE was set at 230 nm. Before each CEC experiment, the OT column was flushed with ultrapure water and the corresponding borax-HCl buffer for 3 min, respectively. All solutions were filtered by 0.45 μm filter membrane, and then sonicated for 4 min for use.

1.6 Determination of binding constants

According to the methods reported[2], 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/mL of His-ZIF-93 dispersion were obtained by dispersing the appropriate amount of His-ZIF-93 in borax-HCl buffer (30 mM, pH = 8.5), respectively. 2 mL of each dispersion was taken and mixed with 2 mL of DTT or DTE (25 μg/mL), respectively. The mixtures were shaken at 150 rpm for 60 min at room temperature. Then, sonicated for 5 min before analysis. The UV absorption spectra from 230 to 320 nm of the mixtures were measured using an UV-1901 spectrophotometer (Persee, China). Benesi-Hildebrand absorption plots for the interaction of

DTT and DTE with His-ZIF-93 were obtained and the binding constants of His-ZIF-93 for DTT and DTE were calculated.

2. Supporting Figures:

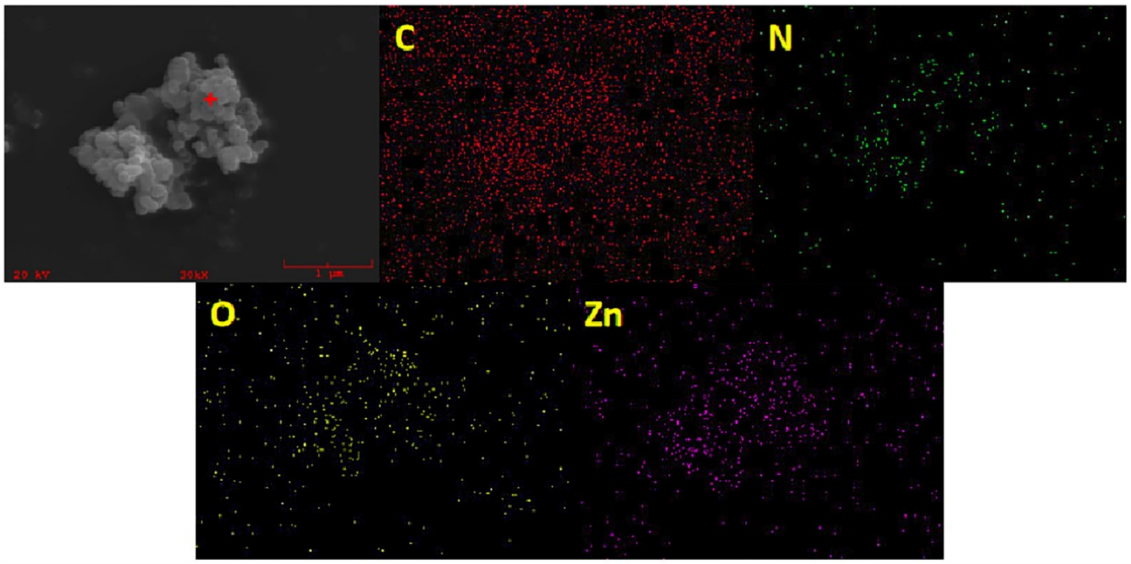

Fig. S1 SEM image and EDS mapping of His-ZIF-93.

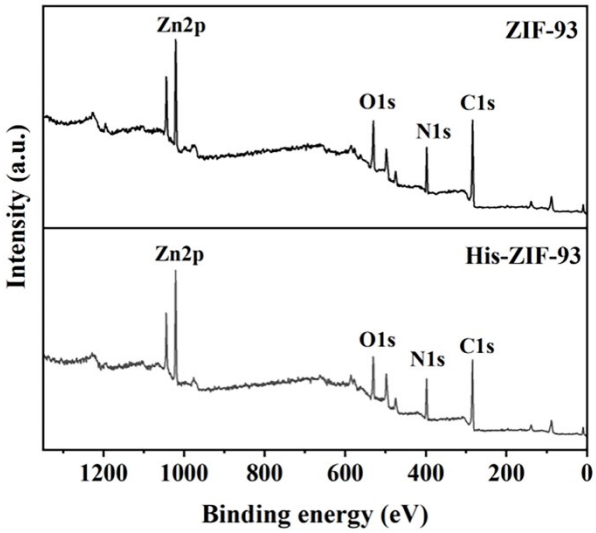

Fig. S2 XPS spectra of His-ZIF-93 and ZIF-93.

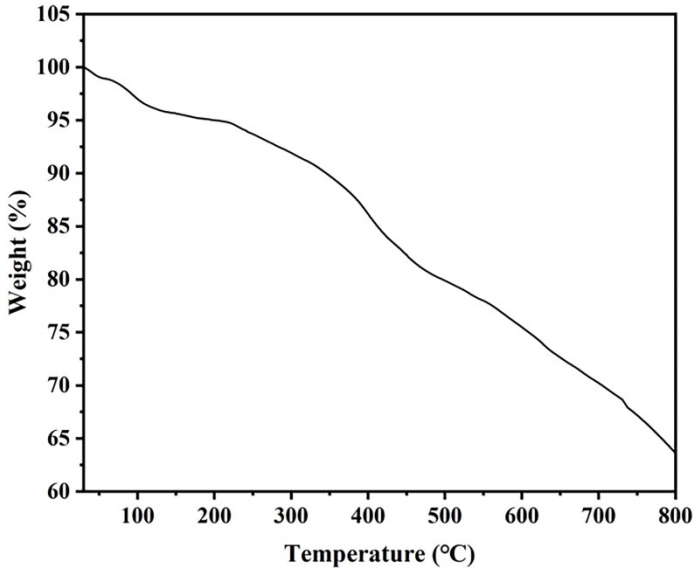

Fig. S3 TGA curve of His-ZIF-93.

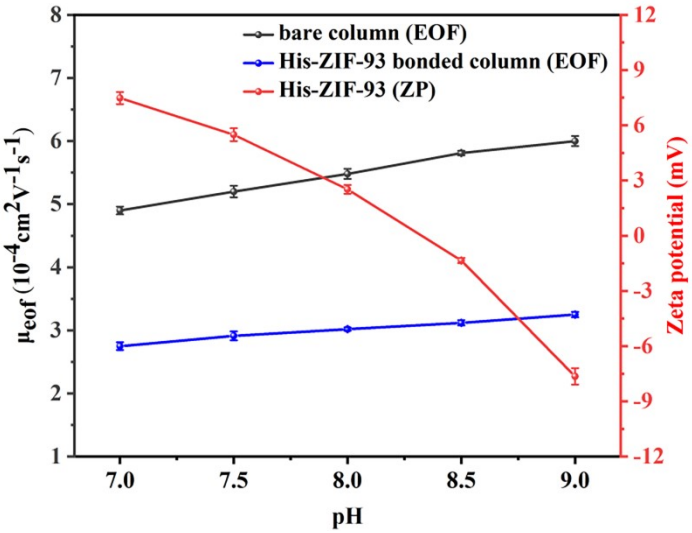

Fig. S4 The effect of buffer pH on EOF (Experimental conditions: sample, 0.1 mg/mL thiourea, 30 mM of borax-HCl buffer, voltage, 15 kV) and ZP curve of His-ZIF-93 (Experimental conditions: 0.2 mg/mL of His-ZIF-93, 30mM borax-HCl buffer).

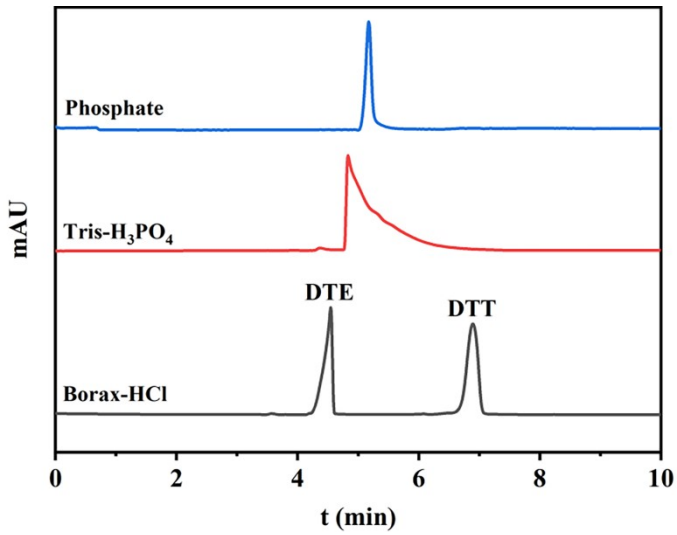

Fig. S5 Effect of buffer types on the separation of DTT and DTE on His-ZIF-93 column (Experimental conditions: 30 mM buffer, pH=8.5, voltage 15 kV, detection wavelength 230 nm).

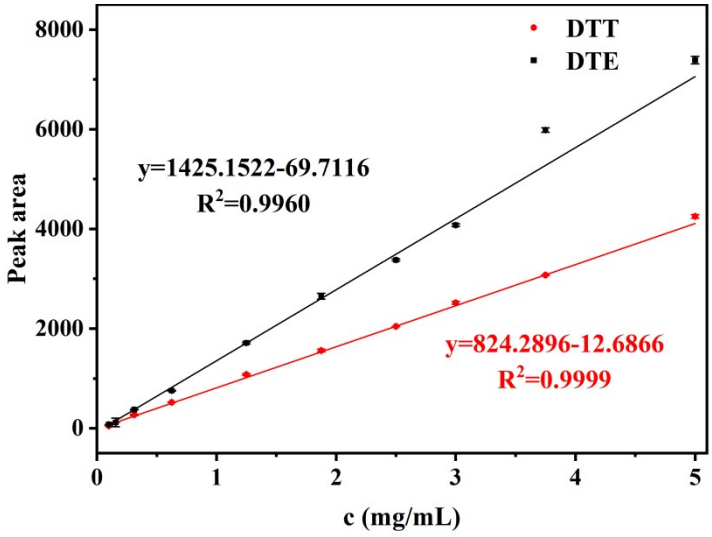

Fig. S6 The standard curves of DTT and DTE (n=5) (Experimental conditions: 30 mM borax-HCl buffer, pH=8.5, voltage 15 kV, detection wavelength 230 nm)

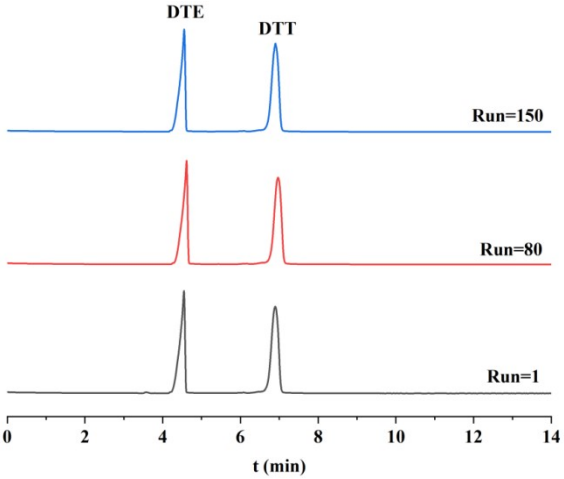

Fig. S7 Separation chromatograms of DTT and DTE with different runs (Experimental conditions: 5.0 mg/mL, 30 mM borax-HCl buffer, pH=8.5, voltage 15 kV, detection wavelength 230 nm).

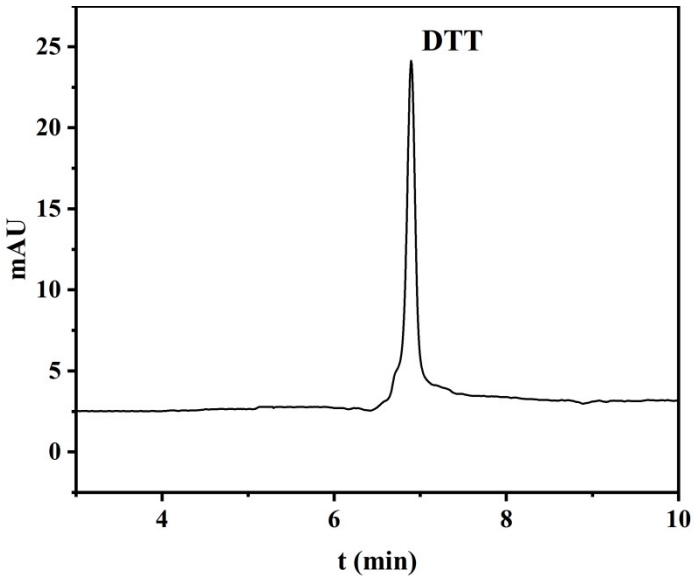

Fig. S8 Electrophorogram of the commercially available DTT sample by His-ZIF-93 column (Experimental conditions: 1.0 mg/mL, 30 mM borax-HCl buffer, pH=8.5, voltage 15 kV, detection wavelength 230 nm).

3. Supporting Tables:

Table S1 The elemental content of His-ZIF-93 and ZIF-93 by EDS

Element	Wt% of His-ZIF-93	Wt% of ZIF-93
C	36.44	37.89
N	18.76	5.19
O	24.67	7.59
Zn	20.13	49.33

Table S2 The specific surface area and pore size of His-ZIF-93 and ZIF-93

Materials	BET（m^2/g）	D（nm）
His-ZIF-93	545	3.99
ZIF-93[3]	864	< 2

Table S3 Binding constants of His-ZIF-93 on DTT and DTE

Stationary phases	Analytes	K (mL/mg)
His-ZIF-93	DTE	49.26
	DTT	51.81

Table S4 Reproducibility and stability of DTT and DTE on His-ZIF-93 OT-CEC column

Type and Numbers	RSD（%）of retention time		RSD（%）of column efficiency	
	DTE	DTT	DTE	DTT
Run-to-run (n = 9)	0.98	1.24	1.78	2.16
Day-to-day (n = 5)	1.78	1.92	2.01	2.23
Column-to-column (n = 5)	1.93	2.07	2.67	2.84
Batch to batch (n=3)	3.10	3.26	3.35	3.44
Runs (n = 150)	2.06	2.45	2.23	2.58

Table S5 Comparison of this work and previous reports on the determination of DTT or DTE

Analytes	Methods	Application	Detection time	LOD	Ref.
DTT	Fluorescent probe	Living cells	10 min	3.1×10^{-7} mol/L	[4]
	Colorimetric method	/	<10 min	0.3 μmol/L	[5]
	Fluorescent probe	/	5 min	0.07 mol/L	[6]
	HPLC-MS	Protein mixtures	23 min	0.1 ng/μL	[7]
	Electrochemical method	A liver extract	<4 min	2.5 μmol/L	[8]
DTE	Optical sensing	Pharmaceutical samples	5 min	0.54 μmol/L	[9]
	HPLC	Pharmaceutical and biological samples	10 min	2.5×10^{-5} mol/L	[10]
DTT, DTE	CEC	Commercial sample Purity test	8 min	0.035 mg/mL for DTT 0.037 mg/mL for DTE	This work

References

[1] M. Gao, J. Wang, Z. Rong, Q. Shi and J. Dong, RSC Advances, 2018, 8, 39627-39634. (DOI: 10.1039/C8RA08460B)

[2] Y. Liu, D. Huang, C. Zhuo, M. Guo, L. Chen, X. Chen, H. Li and W. Xu, Talanta, 2025, 283, 127099. (DOI: 10.1016/j.talanta.2024.127099)

[3] W Morris, B Leung, H Furukawa, OK Yaghi, N He, H Hayashi, Y Houndonougbo and M Asta，Journal of the American Chemical Society, 2010, 132, 11006-11008. (DOI: 10.1021/ja104035j)

[4] C Wang, B Dong, X Kong, N Zhang and W Song and W Lin, Luminescence, 2018, 33, 1048-1053. (DOI: 10.1002/bio.3507)

[5] L Zhao, L Zhao, Y Miao, C Liu and C Zhang, Sensors, 2017, 17, 626. (DOI: 10.3390/s17030626)

[6] S Guo, F Zheng, F Zeng and S Wu, Sensors and Actuators B: Chemical, 2016, 224, 88-94. (DOI：10.1016/j.snb.2015.10.036)

[7] M Cindri , T epo, S Marinc, I Paškvan, I Miji , L Bindila and J Peter-Katalini , Journal of

separation science, 2008, 31, 3489-3496. (DOI: 10.1002/jssc.200800207)

[8] Z. Glatz and P. Ševčíková, Journal of Chromatography B, 2002, 770, 237-241. (DOI: 10.1016/S0378-4347(01)00526-6)

[9] K. Güçlü, M. Özyürek, N. Güngör, S. Baki and R. Apak, Analytica Chimica Acta , 2013, 794, 90-98. (DOI: 10.1016/j.aca.2013.07.041)

[10] M. Özyürek, S. Baki, N. Güngör, S. E. Çelik, K. Güçlü and R. Apak, Analytica Chimica Acta, 2012, 750, 173-181. (DOI: 10.1016/j.aca.2012.03.056)