Supporting information for

Magnetic metal-organic framework nanocomposites for enrichment and direct detection of small molecules by negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry†

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EXPERIMENTAL SECTION

Chemicals and materials. All other chemicals were of analytical grade or better. Seven peptides (Ala-Gln, Gly-Phe, Tyr-Gly-Gly, Tyr-Phe, Phe-Gly-Phe-Gly, Tyr-Gly-Gly-Phe-Leu, and Arg-Ser-Gly-Phe-Tyr) were purchased from Shanghai Apeptide Co. Ltd (Shanghai, China). Amino acids (aspartic acid (Asp), glutamine (Gln), histidine (His), phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp)), hormones (17β-estradiol, testosterone and melatonin), and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 8-Hydroxyquinoline and bisphenol A were the product of Alfa Aesar (Ward Hill, MA, USA). 3-Aminoquinoline (5-AQ) was purchased from Fluka (Buchs, Switzerland). Saturated fatty acids including *n*-dodecanoic acid (C12), *n*-tetradecanoic acid (C14), *n*-hexadecanoic acid (C16), *n*-octadecanoic acid (C18) and *n*-eicosanoic acid (C20) were obtained from Acros Organics (Morris Plains, New Jersey, USA). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate, sodium citrate dehydrate ($Na_3Cit \cdot 2H_2O$), ethylene glycol, and $Zn(NO_3)_2 \cdot 6H_2O$ were obtained from Sinopharm Chemical Reagent, Co., Ltd (Shanghai, China). 2-Methylimidazole (HMeIM) was purchased from J&K Chemical Ltd (Shanghai, China). Methanol (MeOH) and acetonitrile (ACN) were of HPLC grade and supplied by TEDIA (Fairfield, OH, USA). Peptide calibration standard used for calibration of TOF-MS instrument was obtained from Bruker Daltonics (Bruker, Germany). Urine sample was collected from a healthy adult male volunteer. Healthy human serum sample was kindly donated by Fujian Province Official Hospital (Fuzhou, China). Deionized

water (18.2 MΩ cm^{-1}) was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Characterization. Scanning electron microscopy (SEM) images were obtained using an S-4800 TEM (Hitachi, Japan). Transmission electron microscopy (TEM) analyses were performed on a Tecnai G2 20 (FEI, Hillsboro, OR, USA) at 200 kV. The crystal structure of the MMN was determined by X'Pert-Pro MPD (Philips, Holland). Fourier-transform infrared (FTIR) spectra were recorded using a Nicolet 6700 spectrometer (Thermo Fisher, USA) using KBr pellets.

Preparation of Fe_3O_4 microspheres. $FeCl_3$ $6H_2O$ (8.1 g), sodium acetate (12.0 g), Na_3Cit $2H_2O$ (2.0 g) were dissolved in ethylene glycol (200 mL). The obtained homogeneous yellow solution was transferred to autoclave, and then heated to 200 ℃ for 10 h. After reaction, the product was separated with a magnet and washed with water and ethanol for several times.

Synthesis of Fe_3O_4@ZIF-8 MNCs. The Fe_3O_4@ZIF-8 MNCs were prepared according to the our previous work.[1] Briefly, the Fe_3O_4 microspheres (0.35 g) were dispersed in 20 mL of 50% ethanol solution containing 2 mmol $Zn(NO_3)_2$ and 0.2 mmol HCl. Then, 40 mL of 50% ethanol solution containing 20 mmol 2-methylimidazole (HMeIM) was added to above suspension. The resulting mixture was stirred with ultrasound at room temperature for 5 min. The products were collected with a magnet and washed with ethanol and water. In addition, as a control, ZIF-8 MOFs were also synthesized according to the previous work[2].

Preparation of analyte solution. Aspartic acid (Asp), glutamine (Gln), histidine

(His) and phenylalanine (Phe) were respectively dissolved in water at a concentration of 10 mM as stock solutions. Tyrosine (Tyr) and tryptophan (Try) were dissolved in water/formic acid (1:1, v/v) at a concentration of 10 mM at stock solution. All of peptides were respectively dissolved in water/MeOH (1:1, v/v) at a concentration of 5 mM as stock solution. Fatty acids of C12, C14, C16, C18 and C20 were dissolved in anhydrous ethanol at a concentration of 10 mM as stock solutions. 17β-estradiol (E2) and testosterone (T) were dissolved in methanol at a concentration of 10 mM as stock solutions. Melatonin (MT), 8-hydroxyquinoline (8-HQ) and bisphenol A (BPA) were dissolved in anhydrous ethanol at a concentration of 10 mM as stock solutions. All analyte solutions were stored at 4 ℃ for use.

Urine sample used here was collected from healthy adult volunteer. The MT-spiked urine sample was prepared by adding 1 μL of MT (initial concentration, 1.0 mM) into 10 μL urine. Afterward, the above solution was diluted with ACN to 1 mL. Next, an equivalent volume of spiked sample was added to the matrix solution (the final concentration of MT is 0.5 μM) and subsequently vortexed for 30s. Finally, 1 μL of the mixture was pipetted onto the MALDI target plate and air-dried for subsequent MS analysis.

Human serum was collected from healthy adult volunteer at daytime, according to the standard clinical procedures. Then 1pmol MT was directly added into 1 mL of serum and vortexed for 1 min, and then extracted with dichloromethane (5 mL),[3] centrifuged at 10000 rpm for 10 min, and the aqueous phase was discarded, and then the organic phase was evaporated to dryness. The residue was dissolved with ethanol,

and dried again. Afterward, 2 μL of Fe_3O_4@ZIF-8 MNCs were added and subsequently vortexed for 30s. Finally, 1 μL of the mixture was pipetted onto the MALDI target plate and let dry.

Recovery experiment. A known amount of MT standard solution was added to 1mL serum, thereafter all procedures were the same as for the sample solution.

Sample preparation for MALDI-TOF MS analysis. CHCA (10 mg/mL) matrix was dissolved in ACN and 0.1% aqueous TFA at a ratio of 2:1. 3-Aminoquinoline (3-AQ) was dissolved at in ACN/H_2O (1:1) at 10 mg/mL. For the traditional analysis, 1 μL CHCA (or 3-AQ) was first pipetted onto the target plate, and air-dried, followed by 1 μL analyte solution. The matrix of Fe_3O_4@ZIF-8 MNCs were dispersed in ethanol/H_2O (1:1, v/v) and sonicated for 1 min to form homogeneous suspension solutions (2 mg/mL), and then an equivalent volume of analytes was added to the matrix solution and subsequently vortexed for 30s. Finally, 1 μL of the mixture was pipetted onto the MALDI target plate and let dry.

Procedure of sample preparation. Three sample preparation methods for analysis of His were tested before SALDI-TOF MS measurements.

(a) Dried-Droplet Method. 1μL of His (1mM) was added separately to 1× Fe_3O_4@ZIF-8 MNC solutions (1μL). After vortexing for 30s, the resulting mixtures (1μL) were pipetted onto a stainless steel target plate and dried in air.

(b) Matrix-First Method. The 1× Fe_3O_4@ZIF-8 MNC solution (1μL) was deposited first onto the sample plate and let dry. Then, 1μL of His (1mM) was deposited onto the first layer and allowed to dry in air.

(c) Sample-First Method. 1μL of His (1mM) was deposited first onto the sample plate and let dry. Then, 1 × Fe_3O_4@ZIF-8 MNC solution (1μL) was deposited onto the first layer and allowed to dry in air.

Enrichment and analysis of histidine from aqueous solution. MALDI measurement was introduced to detect the enrichment ability of the Fe_3O_4@ZIF-8 MNCs. The procedure was as follows: 1 μL of the Fe_3O_4@ZIF-8 solution (2 mg/mL) was added into 40 μL of His aqueous solution (0.05 μM), the mixture was then vortexed for 30s and kept for enrichment. After standing for 30 min, the Fe_3O_4@ZIF-8 MNCs were isolated with an external magnet and were redispersed in 2 μL of deionized water and 1μL of the above mixture was spotted directly onto the stainless steel plate for the MALDI-MS analysis.

MALDI-TOF MS analysis. MALDI-TOF MS experiments were performed on a Bruker Autoflex Ⅱ mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser operated at 337 nm and laser attenuator offset of ~67% in positive and negative reflection mode. Acquisition parameters included 19 kV acceleration voltages and delayed extraction at 120 ns. Each recorded mass spectrum was generated by averaging data from 500 individual laser shots. To avoid fragmentation of the analytes, the laser power was adjusted to slightly above desorption/ionization (D/I) threshold energy. Mass calibrations were performed externally using the mass peaks of CHCA and 2,5-dihydroxybenzoic acid (DHB) for small molecule (< 500 Da) analysis.

Binding experiments. The adsorption experiments were conducted in a

centrifuge tube at room temperature. His solutions were prepared in binding buffer (10 mM MOPS, pH 7.0). In the isothermal adsorption experiments, 1 mg of Fe_3O_4@ZIF-8 were vortex-mixed with 1 mL of His solution at different concentrations for 30 min in the centrifuge tube at room temperature. After magnetic separation, the supernatants were collected for HPLC-UV analysis. The equilibrium adsorption capacity (Q_e, mg g^{-1}) was calculated according to

$$Q_e = \frac{(C_0 - C_e)}{m} V \quad (1)$$

where C_0 is the initial His concentration (μg mL^{-1}), C_e is the supernatant His concentration (μg mL^{-1}), V is the volume of His solution (mL) and m is the weight of the Fe_3O_4@ZIF-8 (g).

References

(1) Zheng, J.N.; Lin, Z. A.; Lin, G.; Yang, H.H.; Zhang, L. *J. Mater. Chem. B*, 2015, **3**, 2185–2191.

(2) Pan, Y.C.; Liu, Y.Y.; Zeng, G.F.; Zhao, Z.; Lai, Z.P.; Chem. Commun., 2011, **47**, 2071–2073.

(3) Kim, Y.O.; Chung, H.J.; Chung, S.T.; Kim, J.H.; Park, J.H.; Han, S.Y.; Kil, K.S.; Cho, D.H.; *J. Chromatogr. A* 1999, 850, 369-374.

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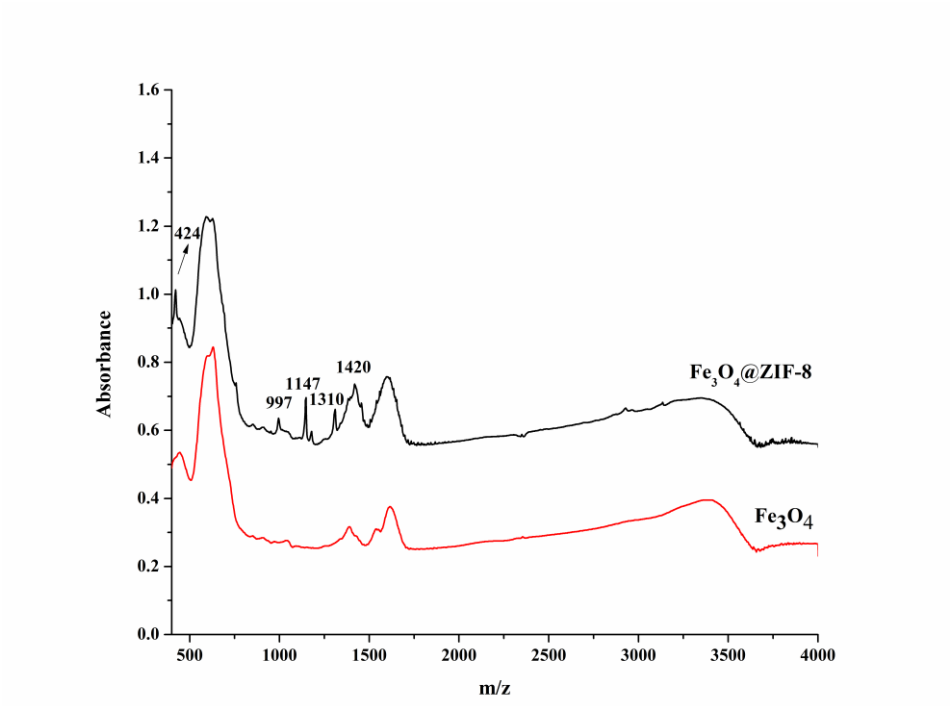

Fig.S1(A) FT-IR spectra of the Fe_3O_4@ZIF-8 MNC and bare Fe_3O_4.

FT-IR spectra of Fe_3O_4@ZIF-8 and Fe_3O_4 were presented in Fig.S1, Compared to the spectrum of Fe_3O_4 microspheres, the spectrum of Fe_3O_4@ZIF-8 displays additional absorption adsorption bands which are associated with the ZIF-8 structure. The board band centered at 1420 cm^{-1} and bands in the spectral region of 900–1330 cm^{-1} could be assigned to the stretching and plane bending of imidazole ring, respectively. The band at 424 cm^{-1} is attributed to the characteristic Zn–N stretch mode. Overall, the FT-IR spectra also confirmed the formation of ZIF-8 shells.

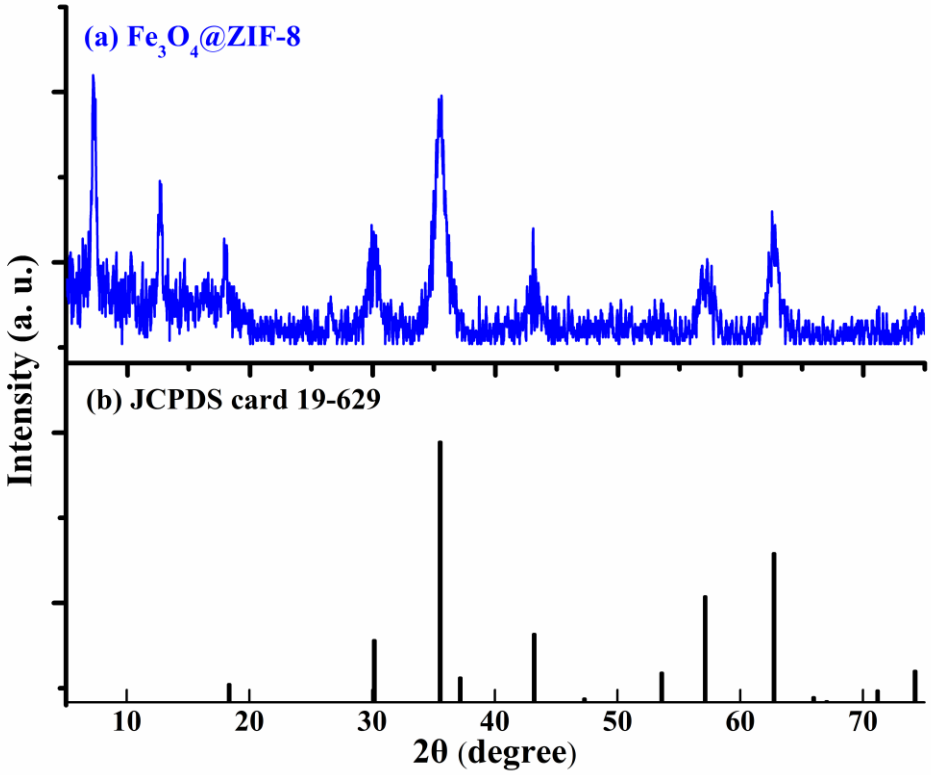

Fig. 1(B) (a) XRD pattern of Fe_3O_4@ZIF-8; and (b) the standard diffraction lines of Fe_3O_4 (JCPDS card 19-629)

X-ray diffraction (XRD) measurement confirmed the crystalline structure of Fe_3O_4@ZIF-8 MNCs. The XRD pattern of Fe_3O_4@ZIF-8 can be assigned to a superposition of characteristic peaks of face-centered cubic Fe_3O_4 (JCPDS card 19-629) and simulated pattern of the published ZIF-8 structure data.[4]

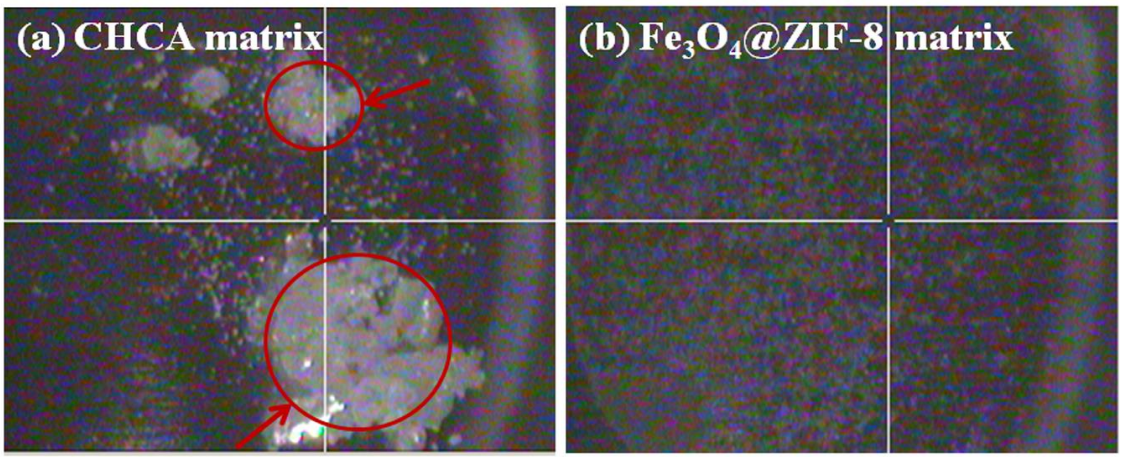

Fig.S2 Optical images of CHCA and Fe_3O_4@ZIF-8 MNCs dispersed on the stainless steel target. Matrix concentration: CHCA (10 mg/mL) (Sweet spots in red circles) and Fe_3O_4@ZIF-8 MNCs (1 mg/mL).

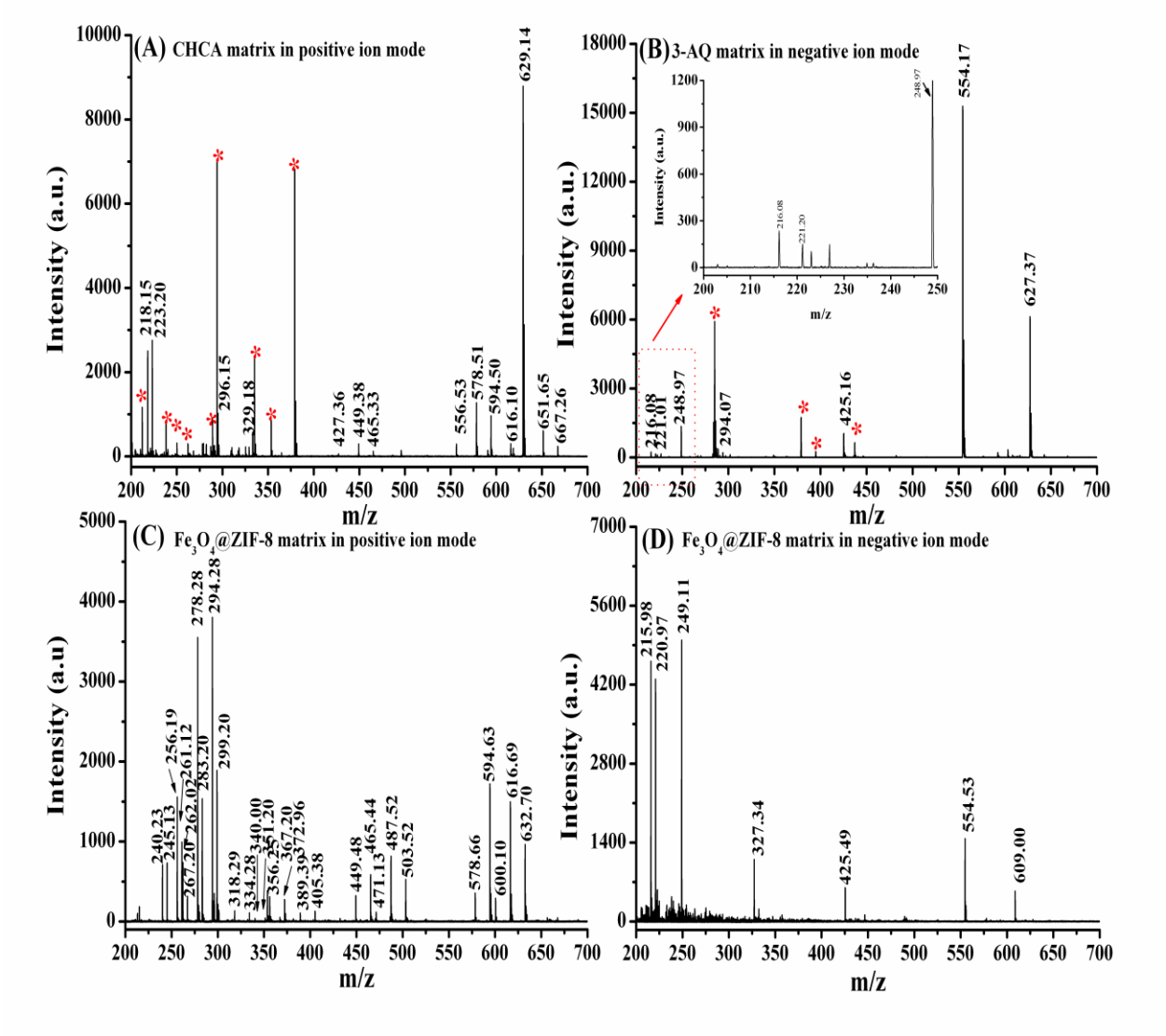

Fig.S3 Mass spectra of peptides by using different matrices. (A) CHCA matrix in positive ion mode; (B) 3-AQ matrix in negative ion mode; (C) Fe_3O_4@ZIF-8 MNC matrix in positive ion mode; (D) Fe_3O_4@ZIF-8 MNC matrix in negative ion mode. The concentrations of Ala-Gln (0.5 mM), Gly-Phe (1 mM), Tyr-Gly-Gly (0.3 mM), Tyr-Phe (0.3 mM), Phe-Gly-Phe-Gly (0.25 mM), Tyr-Gly-Gly-Phe-Leu (0.32 mM) and Arg-Ser-Gly-Phe-Tyr (0.29 mM) were used for Fe_3O_4@ZIF-8 MNC matrix and two-fold concentrations of all peptides for CHCA or 3-AQ matrix. The same laser intensity of 65% was applied for all.

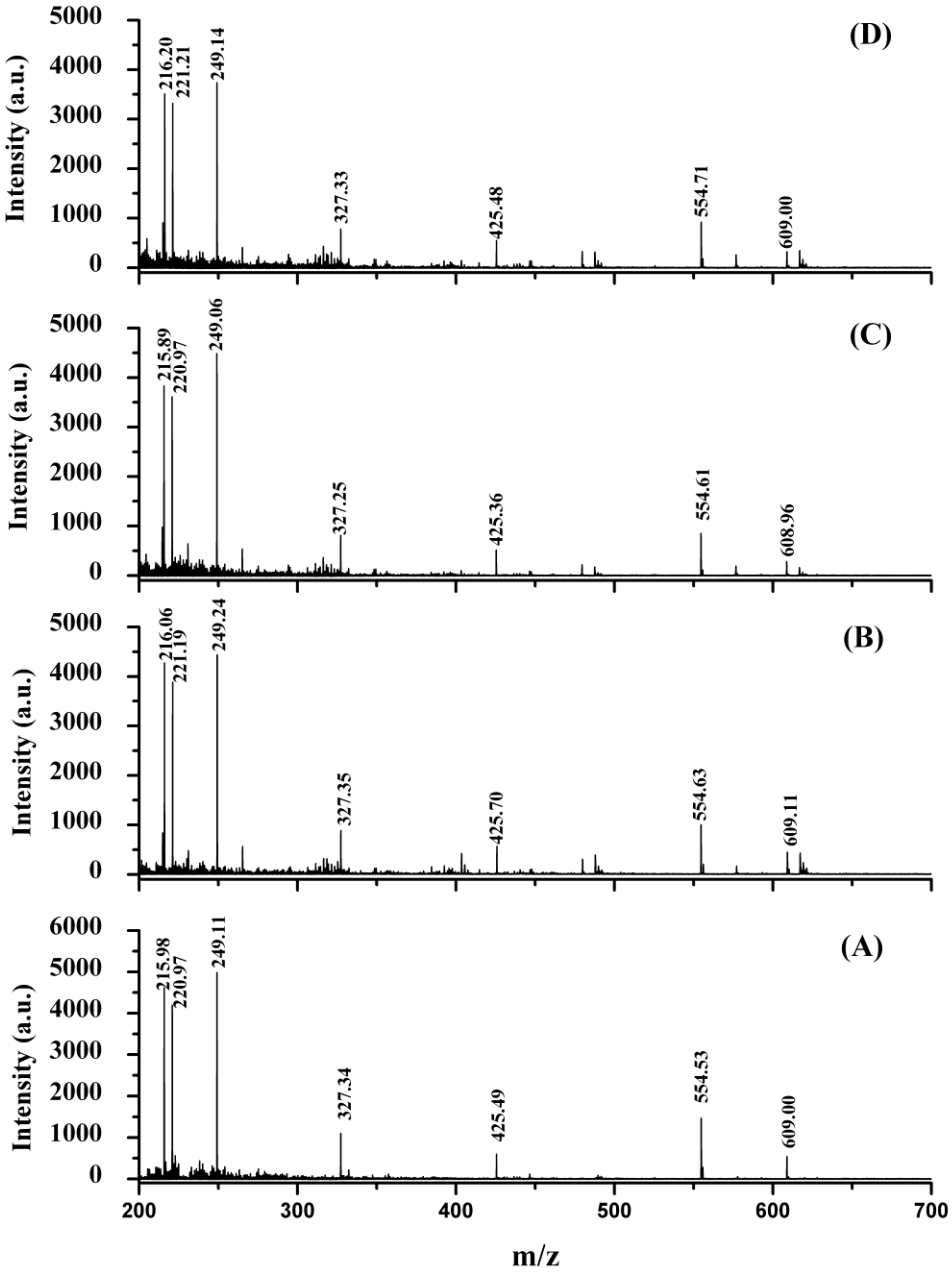

Fig.S4. Mass spectra of peptide by using Fe_3O_4@ZIF-8 MNC matrix in negative ion mode with no additional salt (A); 10 mM NaCl (B); 100 mM NaCl (C); 1000 mM NaCl (D). All the experimental conditions were same as Fig.S3.

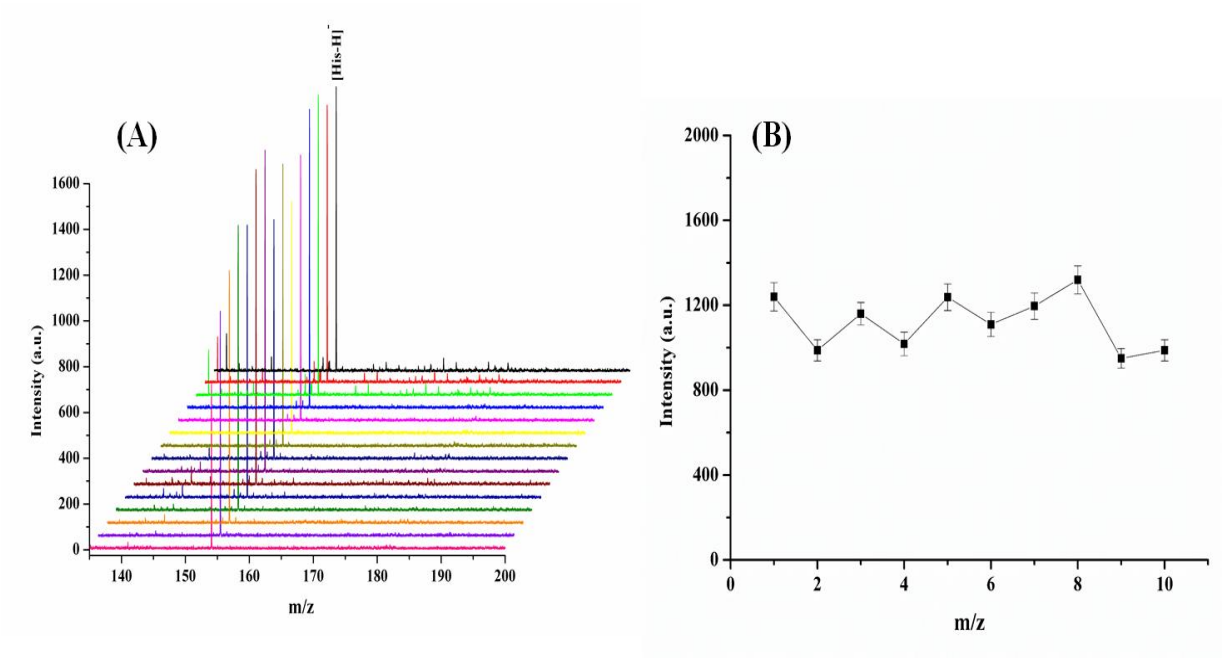

Fig.S5. MS signal intensity of His repeatedly acquired from one sample spot (A) and from 10 different sample spots (B) by using Fe_3O_4@ZIF-8 MNC matrix in negative ion mode. The concentration of His was 0.5 mM. All the analysis was performed under the same experimental condition (laser intensity of 60%)

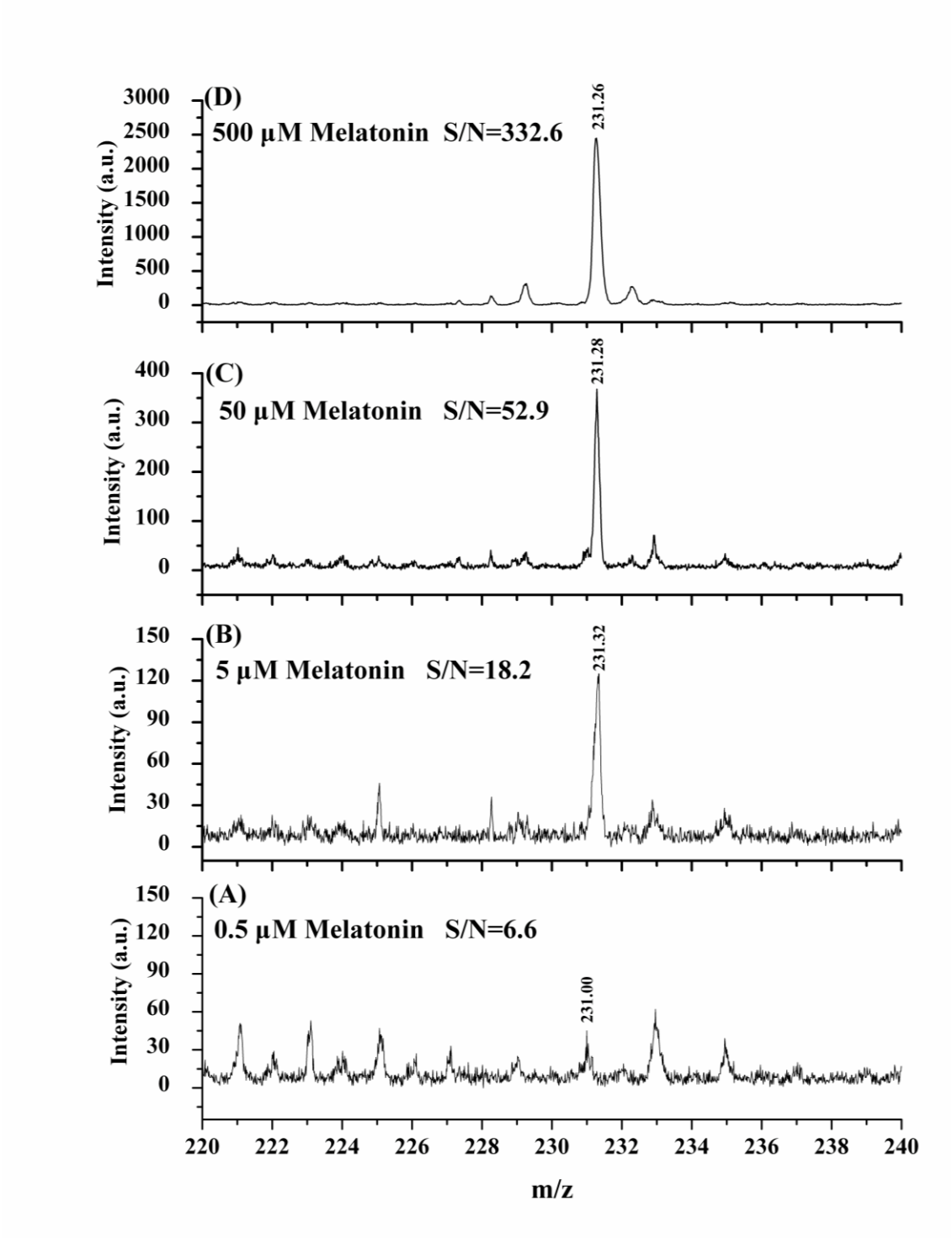

Fig.S6 Mass spectra of MT at different concentrations (0.5 µM, 5 µM, 50 µM, and 500 µM) by using Fe_3O_4@ZIF-8 MNC matrix in negative ion mode. The same laser intensity of 65% was applied for all.

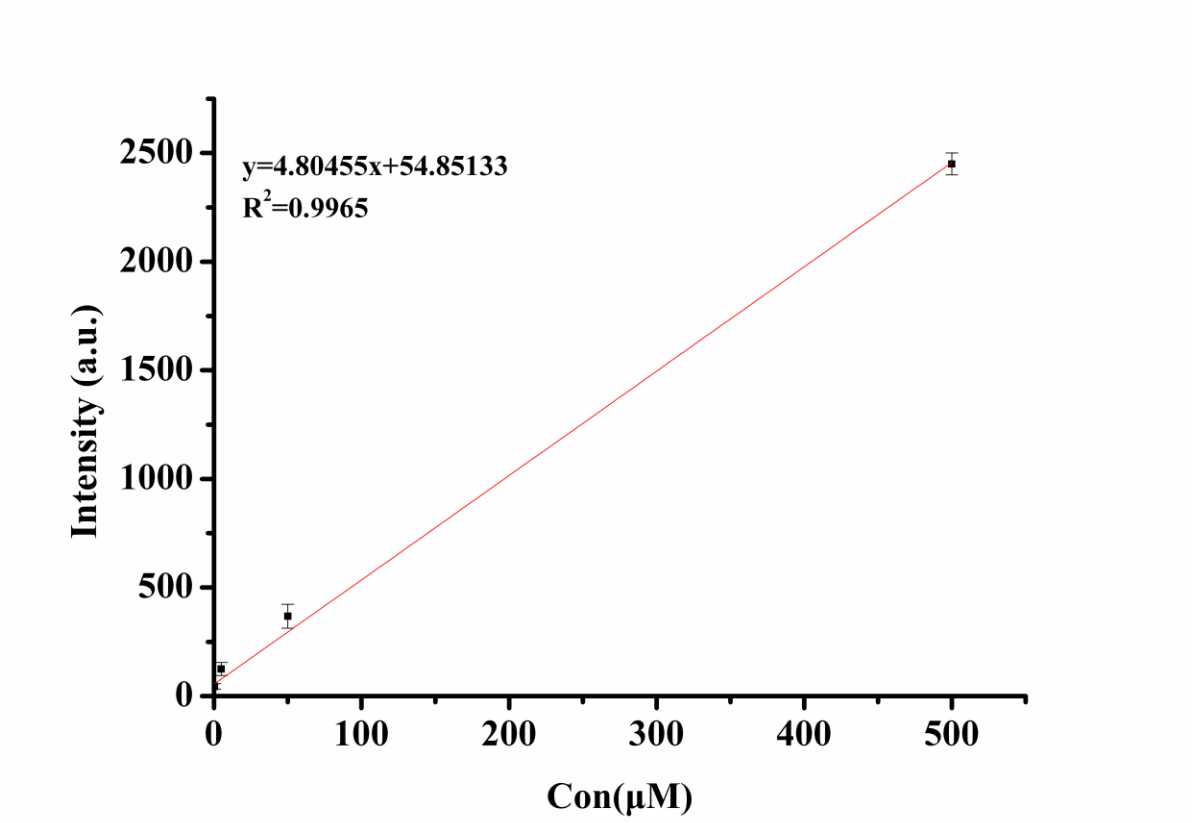

Fig.S7. The calibration curve of MT obtained by MALDI-TOF MS using Fe_3O_4@ZIF-8 MNC matrix.

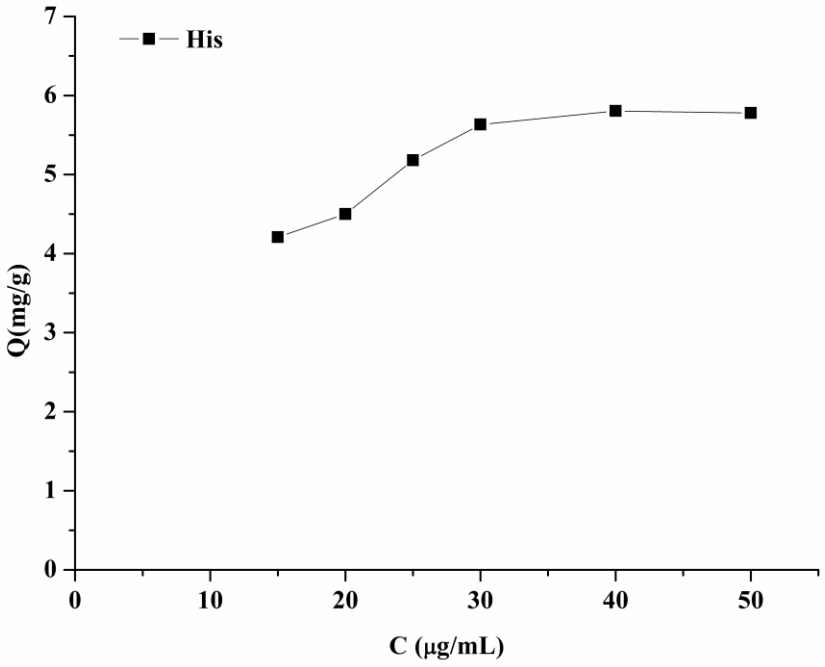

Fig.S8 Adsorption isotherms of His on the Fe_3O_4@ZIF-8 MNCs

Amount of Fe_3O_4@ZIF-8 MNCs: 1 mg; volume: 1 mL; incubation time: 30 min;

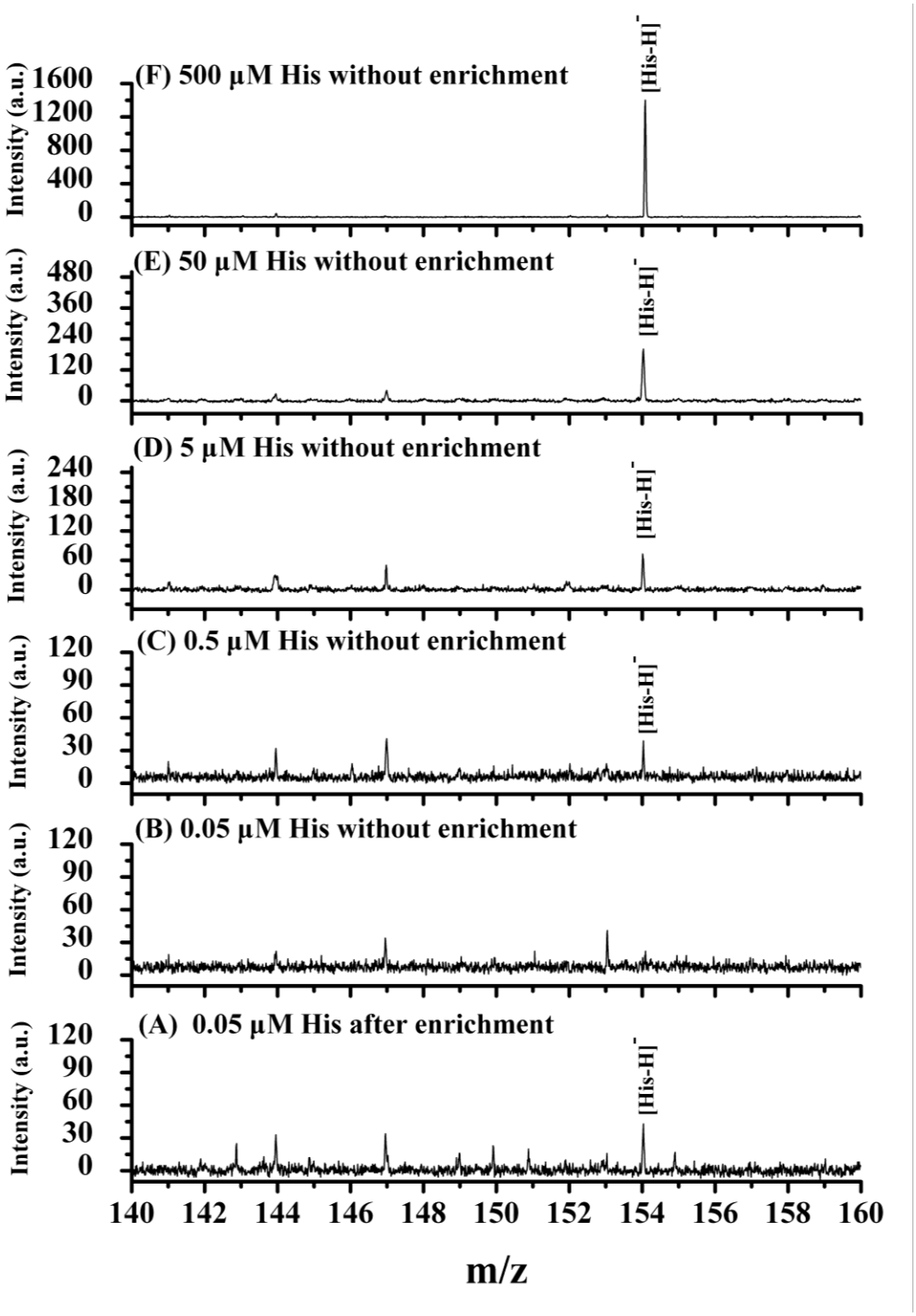

Fig.S9. Mass spectra of different concentrations of His in water solution with Fe_3O_4@ZIF-8 MNCs as adsorbent and matrix. The same laser intensity of 60% was applied for all.

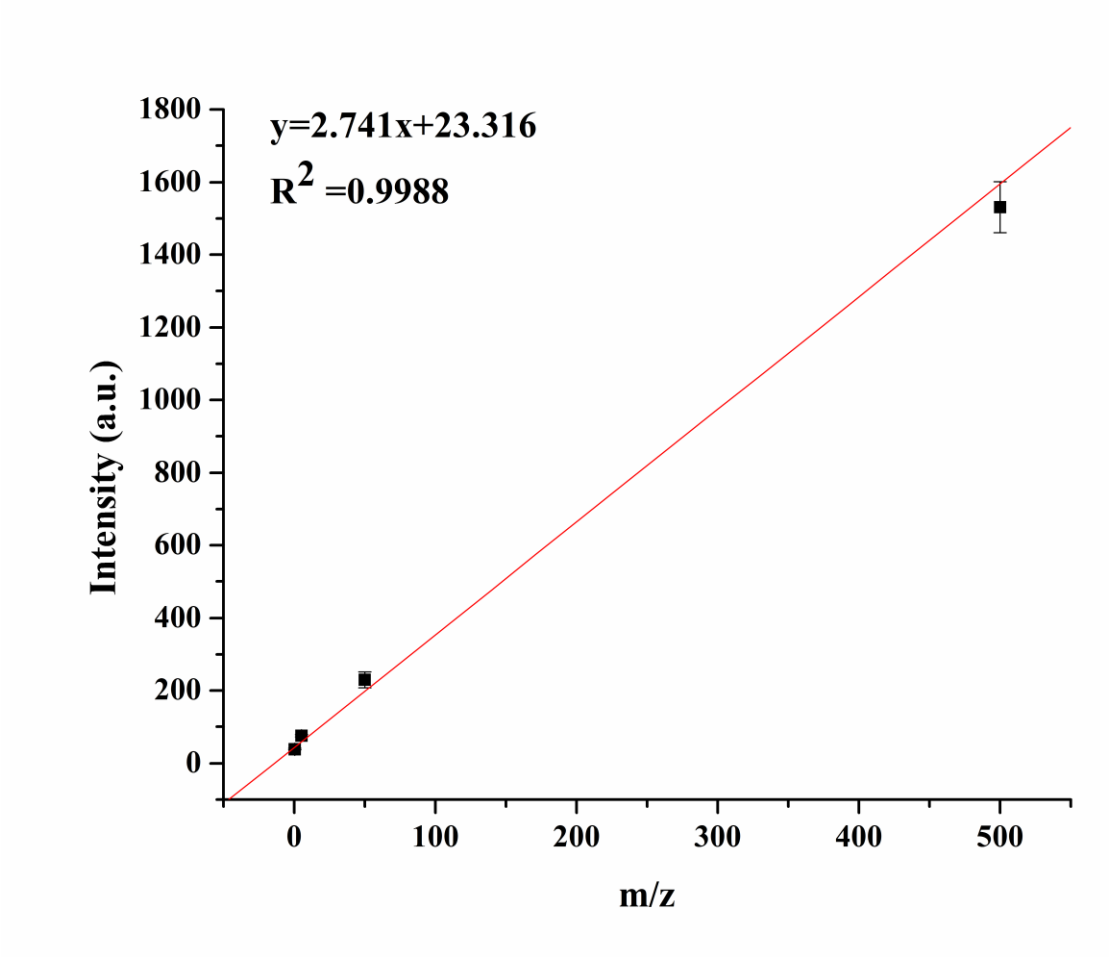

Fig.S10 The calibration curve of His obtained by MALDI-TOF MS using Fe_3O_4@ZIF-8 MNC matrix.

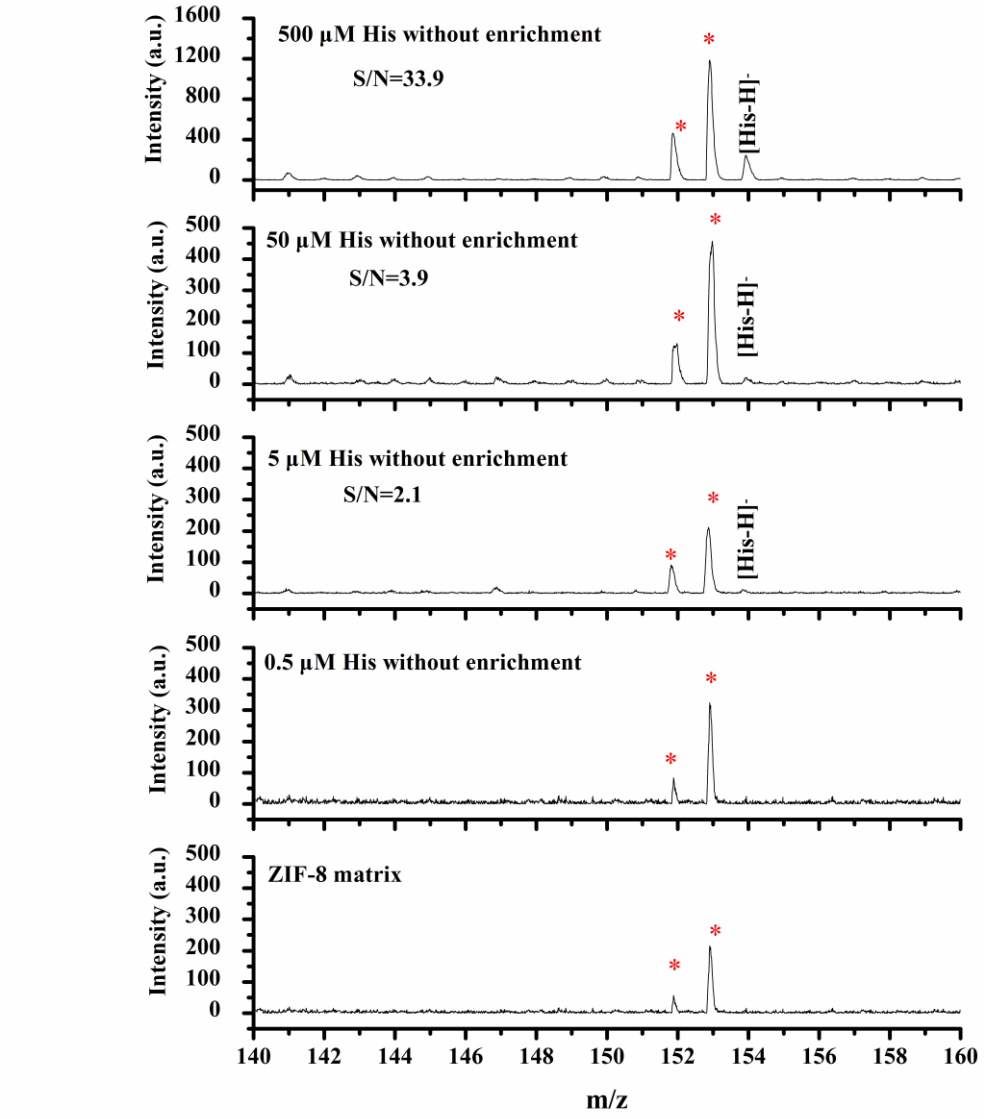

Fig.S11 Negative ion MALDI-TOF MS for analysis of His using ZIF-8 as adsorbent and matrix.

The experimental conditions are same as Fig.S9

Table S1 Peak identification of amino acids for positive-ion LDI using Fe_3O_4@ZIF-8 MNCs (Fig. 2(B))

Compounds	M	$[M+Na]^+$	$[M+K]^+$	$[M+2Na-H]^+$	$[M+Na+K-H]^+$	$[M+2K-H]^+$
Asp	133.11	156.15	172.17	178.06	194.04	210.10
Gln	146.14	169.05	185.01	N	207.10	223.00
His	155.15	178.06	194.04	N	216.03	232.01
Phe	165.19	188.09	204.00	210.10	226.00	241.99
Tyr	181.19	204.00	220.00	226.00	241.99	257.99
Trp	204.23	227.09	243.07	N	265.02	281.05

*N represents not observed

Table S2 Peak identification of peptides for positive-ion LDI using Fe_3O_4@ZIF-8 MNCs (Fig.S3(C))

Compounds	M	$[M+Na]^+$	$[M+K]^+$	$[M+2Na-H]^+$	$[M+Na+K-H]^+$	$[M+2K-H]^+$
Ala-Gln	217.22	240.23	256.19	262.02	278.28	294.28
Gly-Phe	222.24	245.13	261.12	267.20	283.20	299.20
Tyr-Gly-Gly	295.12	318.29	334.28	340.00	356.25	372.36
Tyr-Phe	328.14	351.20	367.20	372.96	389.39	405.38
Phe-Gly-Phe-Gly	426.19	449.48	465.44	471.13	487.52	503.52
Tyr-Gly-Gly-Phe-Leu	555.27	578.66	594.63	600.10	616.69	632.70
Arg-Ser-Gly-Phe-Tyr	628.69	N	N		N	N

*N represents not observed

Table S3. Determination of MT in human serum and recovery test (n=3).[a]

Compound	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
MT	**1.0**	**4.46**	**89.2**	**6.8**
	10.0	**9.26**	**92.6**	**5.6**

a: Experimental conditions are the same as in Fig. 4.