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Magnetic molecularly imprinted polymer for the selective extraction of sildenafil, vardenafil and their analogs from herbal medicines

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ABSTRACT

The successfully developed magnetic molecularly imprinted polymers (MMIPs) toward six synthetic phosphodiesterase type-5 (PDE-5) inhibitors were described. Sildenafil was used as template for the preparation of MMIPs using superparamagnetic core–shell nanoparticle as supporter. The obtained MMIPs were characterized using transmission electron microscope, Fourier transform infrared, X-ray diffraction, and vibrating sample magnetometer. High performance liquid chromatography (HPLC) with diode array detector (DAD) was used for the analysis of target analytes. The application of MMIPs as selective sorbent in the cleanup of herbal medicine samples prior to HPLC offered simple sample preparation. The adsorption capacity and selectivity of prepared MMIPs and magnetic non-molecularly imprinted polymers were investigated. The binding isotherms were obtained for sildenafil and fitted by Freundlich isotherm model. Structurally similar compound of sildenafil and a reference compound protocatechuic acid were used for investing the selective recognition of MMIPs.

1. Introduction

Sildenafil citrate (Viagra, Pfizer), vardenafil hydrochloride (Levitra, Bayer) and tadalafil (Cialis, Elli Lilly), which are synthetic phosphodiesterase type-5 (PDE-5) inhibitor drugs, are marked as approved drugs for the treatment of erectile dysfunction in males. The advent of these highly successful drugs has spurred the making of herbal alternatives for the enhancement of sexual performance. In recent years, these herbal alternatives have been found containing synthetic PDE-5 inhibitors as adulterants [1–4], such as sildenafil, vardenafil, tadalafil, methisosildenafil, etc. Although three PDE-5 inhibitors approved by the European Medicines Agency (EMA) and Food and Drug Administration (FDA) appear to be relatively safe, their illicit usage is still worrying because of harmful side-effects such as headache, flush, dyspepsia, rhinitis, back pain [5]. However, there are reports that even the unapproved synthetic analogs of PDE-5 inhibitors have been found in herbal dietary supplements [3,4,6] such as homosildenafil, hydroxyhomosildenafil, methisosildenafil, acetildenafil, etc. The illegal products may have risk for public health. The reports on detection and identification of PDE-5 inhibitor analogs in herbal medicines include the compounds such as homosildenafil [7–10], hydroxyhomosildenafil [7,10], acetildenafil [7,11,12], aminotadalafil [8,13], pseudo-vardenafil [13,14], and piperadino acetildenafil [13], etc.

Recently, several articles had eloquently described analytical techniques and strategies for detection and identification of PDE-5 inhibitors as adulterants in herbal medicines. The techniques such as LC–MS [1,7,8,12,14], LC–MS/MS [2], capillary electrophoresis [15], and the combination of various methods [8] were the most common methods. The presence of complex matrix interference leads to peak suppression or reducing sensitivity in many cases. In all of these methods, they adopted the traditional sample processing methods, which were not selective. It is important to develop a selective and practicable enrichment material for investigation and determination of PDE-5 inhibitors in drug-safety field. Molecular imprinted polymers are functional porous materials with molecular specific recognition sites to a particular target molecule similar to the results obtained by immune affinity columns [16–18]. They have been widely applied in the field of selective determination of target molecules, such as the extract of traditional Chinese medicines [18], food and environmental media [19], biological sample [20], etc. The magnetic molecularly imprinted polymer (MMIPs) can be dispersed into the solution directly and then easily separated from the matrix using an external magnetic field without additional centrifugation or filtration [21–23]. A successfully MMIPs toward tadalafil (TD) was developed by Li et al. [22] which used TD as the template

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molecule. However, the selectivity toward the unapproved synthetic analogs of PDE-5 inhibitors was not tested. MMIPs containing sildenafil binding sites was produced by Ding et al. [23]. The recoveries were only determined for sildenafil and vardenafil (VD). In this work the MMIPs were developed and applied as a sorbent in solid-phase extraction protocol for the group detection of PDE-5 inhibitors in herbal medicines. This approach was chosen as an alternative cleanup procedure in place of the commonly used extensive procedure involving liquid–liquid extractions, centrifuging, and solid-phase extractions.

To our knowledge, MMIPs have not yet been developed for the group detection of PDE-5 inhibitors in herbal medicines. The present study elaborates on the successfully developed MMIPs toward six synthetic PDE-5 inhibitors. Sildenafil was used as the template for preparation of MMIPs using superparamagnetic core–shell nanoparticle as supporter. The adsorption capacity and selectivity of the MMIPs and magnetic non-molecularly imprinted polymers (MNIPs) were investigated. The application of MMIPs as sorbent in an SPE procedure was validated for selective extraction of sildenafil, VD, and their analogs from herbal medicines.

2. Experimental

2.1. Reagents and apparatus

Sildenafil was purchased from Zhengzhou Lion Biological Technology Co., Ltd., protocatechuic acid (PA), aminotadalafil (ATD), vardenafil (VD), homosidenafil (HSD), pseudovardenafil (PVD), acetildenafil (AD), hydroxyhomosidenafil (HHS), and tadalafil (TD) were provided by the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Trimethylolpropane trimethacrylate (TRIM) and divinylbenzene (DVB) were purchased from TCI (Shanghai) Development Co., Ltd.. Ethylene glycol dimethacrylamide (EGDMA), acrylamide, methacrylic acid (MAA), 3-methacryloxypropyltrimethoxysilane (MPS), and 2,2′-azobisissobutyronitrile (AIBN) were obtained from Alfa Aesar (Tianjin, China). Anhydrous toluene, ammonium acetate, and ethanol were purchased from Lianlong Bohua (Tianjin) Pharmaceutical Chemical Co., Ltd (Tianjin, China). Chromatographic grade methanol was purchased from Merck Co. (Darmstadt, Germany). Isopropanol, acetic acid, and the other chemicals were supplied from Tianjin Chemical Reagent Co. (Tianjin, China). Deionized water (18 MΩ cm) was prepared with a water purification system (Shanghai, China). Herbal health products were purchased from the market and the manufacturer was in Shanxi.

TEM images were obtained via a Tecnai-G2-F30 field emission transmission electron microscope (TEM, FEI, USA). The Fourier transform infrared (FT-IR) spectra were obtained via a Nicolet Nexus-670 FT-IR spectrometer. The wave numbers of FT-IR measurement range were controlled from 500 cm^{-1} to 4000 cm^{-1}. The magnetic properties were measured using Lake Shore 7304 vibrating sample magnetometer (VSM) (Lakeshore, USA). X-ray diffraction (XRD) pattern was carried out by an X-ray diffraction using Cu-Ka1 radiation (PANalytical X'Pert, Holland). Sample analysis was performed using liquid chromatographic system equipped with Agilent 1200 HPLC system and diode array detection (DAD) system. The analytical column was a 250 mm × 4.6 mm, 5 μm C_{18} column (Agilent, USA). The mobile phase was consisted of methanol and 15 mM ammonium acetate buffer with the linear gradient elution 0–15 min for 65–80% methanol at a flow rate of 1.0 mL min^{-1}. DAD monitoring was at 230 nm and 20 μL of sample was injected.

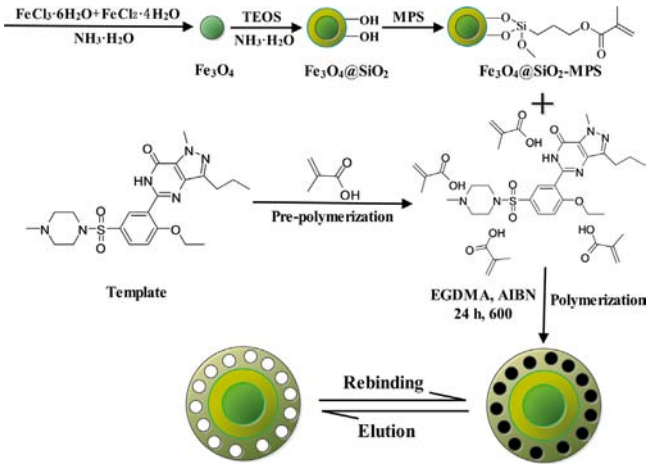

Fig. 1. Schematic representation of the possible process of sildenafil MMIPs.

2.2. Preparation of MMIPs

The preparation protocol was shown in Fig. 1. Fe_3O_4@SiO_2–MPS nanoparticles were first prepared. The Fe_3O_4 particles were prepared using chemical co-precipitation according to Chen et al. [21]. Then Fe_3O_4 particles were modified with SiO_2 according to the work of Zeng et al. [24]. The obtained Fe_3O_4@SiO_2 nanoparticles were dried under vacuum at 60 °C, and modified with MPS introduced polymerizable double bonds [25]. Briefly, 1 g of Fe_3O_4@SiO_2 nanoparticles were dispersed in 50 mL of anhydrous toluene containing 5 mL of MPS, and the mixture reacted with reflux at 90 °C for 24 h under dry nitrogen. The products were collected and washed with toluene and ethanol for several times. Finally, surface-modified magnetic particles (Fe_3O_4@ SiO_2–MPS nanoparticles) were dried under vacuum at 60 °C.

Sildenafil (0.2 mmol) as the template and MAA (0.8 mmol) as the functional monomer were dissolved into 36 mL toluene and stored in dark for 12 h at room temperature. Then, 200 mg of Fe_3O_4@SiO_2–MPS nanoparticles was added into the mixture, stirring for 2 h. Subsequently, cross-linker EGDMA (3.2 mmol) and initiator AIBN (40 mg) were added into the system and the mixture was degassed in an ultrasonic bath for 15 min. After filled with nitrogen gas for 10 min to remove oxygen, the polymerization was performed at 60 °C with nitrogen protection for 24 h. The MMIPs were collected magnetically, and washed by a mixture of methanol/acetic acid (9:1, v/v) to remove the templates and then washed by methanol until no sildenafil absorption was detected by HPLC. Finally, the particles were dried in vacuum. The MNIPs were prepared by the same method as MMIPs without the addition of template.

2.3. Binding experiment

MMIPs or MNIPs of 20 mg were added into centrifuge tubes, respectively, and were mixed with sildenafil standard solution (0.01–1 μmol mL^{-1}) in acetonitrile. After the samples were shaken in SHA-B incubator (Jintan Zhengji Instrument Co., Ltd., Jiangsu, China) for 24 h at 25 °C, a magnet was deposited at the outside of the tube to separate MMIPs or MNIPs from the solution, and then the supernatant was measured using HPLC. The amount of sildenafil binding to the MMIPs or MNIPs was calculated by subtracting the amount of free sildenafil from the amount of sildenafil initially added. The data of the absorption experiment was further processed according to the Freundlich isotherm (FI) model to estimate the binding parameters of the MMIPs and MNIPs.

2.4. Specificity of MMIPs

The specificity of the magnetic imprinted sorbent was investigated with HSD, HHS, PVD, ATD, AD, VD, and TD as structural analogs of sildenafil template, and PA as reference compound. One milliliter of a 0.01 μmol mL^{-1} solution prepared in acetonitrile was incubated with 20 mg MMIPs or MNIPs. In order to further investigate the competitive recognition coefficients of the sorbents, PA was chosen as reference for measurement in a mixed solution of sildenafil and PA (0.01 μmol mL^{-1} for each). The extraction procedure was then conducted as described earlier in binding experiment.

2.5. Extraction procedure

Six hundred milligram of each pulverized samples mixed with 10 mL methanol was extracted by sonication for 20 min. The extract was filtered and 1 mL of filtrate spiked with the standards mixture was evaporated to dryness under nitrogen gas at room temperature. Fifty milligram of MMIPs was added to the residues and dissolved in 1 mL of acetonitrile, shook at room temperature for 20 min. A magnet was used to separate MMIPs from the solution. After the supernatant solution was discarded, the MMIPs were washed with 1 mL acetonitrile. Finally, the MMIPs were eluted with 1 mL of methanol/acetic acid (9:1, v/v) by sonication for 15 min [17,21]. 0.8 mL supernatants were completely evaporated and dissolved in 0.08 mL of methanol for a further HPLC-DAD analysis.

3. Results and discussion

3.1. Preparation of MMIPs

The MMIPs applied in determination of PDE-5 inhibitors were prepared using TD as template molecule [22], and high recovery of TD had been achieved. The sildenafil was used as template molecule, and higher recoveries can be obtained for sildenafil and VD by sildenafil MMIPs according to the report [23]. In our study, sildenafil was also selected as template molecule. Firstly, Fe_3O_4 nanoparticles were coated with silica for obtaining the biocompatible and hydrophilic shell, and the surface silanol groups could be further modified through covalent attachment of MPS [26]. During the synthesis procedure of the MMIPs, the introduced double bonds by MPS on the Fe_3O_4@SiO_2 nanoparticles can react with functional monomer and cross-linker [27]. The generation of recognition sites was dependent on the functional monomer through organized self-assembly with the template; thus, the same amounts of MAA and acrylamide were tested for monomer selection. The resulting polymers prepared using MAA had better molecular recognition for sildenafil. The cross-linker can affect the degree of cross-linking of the polymeric network. Thus, EGDMA, TRIM, DVB, and mixture of two cross-linker compounds (1:1, molar ratio) were tested as cross-linkers in the MMIPs preparation. The resulting polymers prepared using EGDMA had better uniformity structure than either TRIM, DVB, or the mentioned mixed cross-linkers did. The polymers prepared in the study were stable and magnetic. The RSD% of batch-to-batch reproducibility of the prepared MMIPs was 8.28%, showing that the synthesis process was satisfactory.

3.2. Characterization of MMIPs

Morphological features of Fe_3O_4@SiO_2 and MMIPs were characterized by TEM. The Fe_3O_4@SiO_2 is of uniform spherical morphology with about 500 nm in size (Fig. 2A). The MMIPs still revealed the fine spherical morphology with the diameter of 800–1000 nm (Fig. 2B). The size became larger than Fe_3O_4@SiO_2, which was a result of imprinted layer formation.

During the application of magnetic particles in fast separation, magnetic property was the key factor. The magnetic properties of synthesized Fe_3O_4, Fe_3O_4@SiO_2, and MMIPs were estimated by VSM. The saturation magnetizations are 73.73, 53.56, and 41.16 emu g^{-1} for Fe_3O_4 nanoparticles, Fe_3O_4@SiO_2, and MMIPs, respectively (Fig. 3). The polymeric coating on the surface of Fe_3O_4@SiO_2 and MMIPs slightly shields magnetic force, and the saturation magnetization of them was a little less than that of Fe_3O_4. As a result of enough magnetism, the MMIPs could be rapidly magnetic separation with an external magnetic field.

The FT-IR spectra of Fe_3O_4, Fe_3O_4@SiO_2, Fe_3O_4@SiO_2–MPS, MMIPs, and MNIPs are shown in Fig. 4. The absorbance peak around 800, 954, and 1100 cm^{-1} attributed to the stretching of Si–O, Si–O–H and Si–O–Si, respectively (Fig. 4a). In Fig. 4b, the peak at 2924 cm^{-1} was assigned to the C–H stretching vibration in methylene group, which indicated that MPS was successfully modified on the surface of Fe_3O_4@SiO_2 nanoparticles. In Fig. 4c and d, MMIPs and MNIPs showed similar locations of the major bands. The broad absorption peak at 3440 cm^{-1} of MMIPs and MNIPs, corresponded to O–H bonding to hydroxyl groups for MAA (functional monomer). The peak around at 579 cm^{-1} represented the existence of Fe_3O_4. These results confirmed that Fe_3O_4

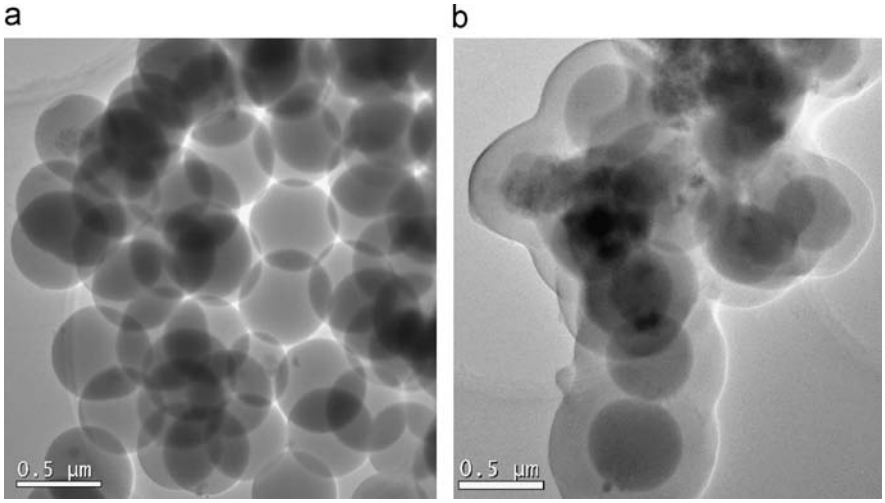

Fig. 2. Transmission electron micrographs of the Fe_3O_4@SiO_2 (A) and MMIPs (B).

nanoparticles were successfully packed into the polymers, and the formation of imprinted and non-imprinted layers on Fe_3O_4@SiO_2 was successful.

The components of Fe_3O_4, Fe_3O_4@SiO_2, and MMIPs were analyzed using XRD (see supplementary material). Similar peaks were displayed in the 2θ region of 10–80°, indicating that the synthesis process did not change the XRD phase of Fe_3O_4.

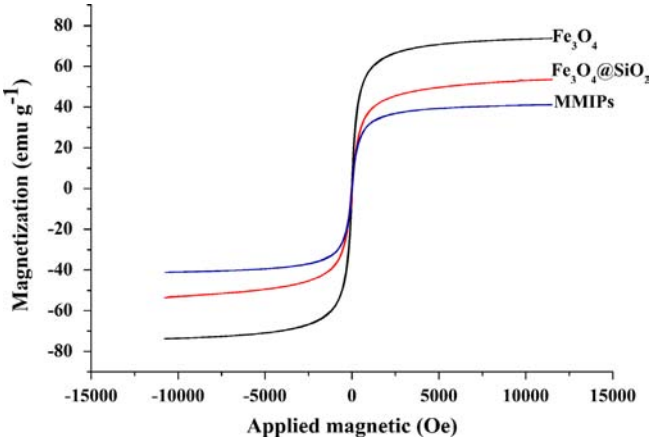

Fig. 3. Hysteresis loops of Fe_3O_4 nanoparticles, Fe_3O_4@SiO_2, and MMIPs.

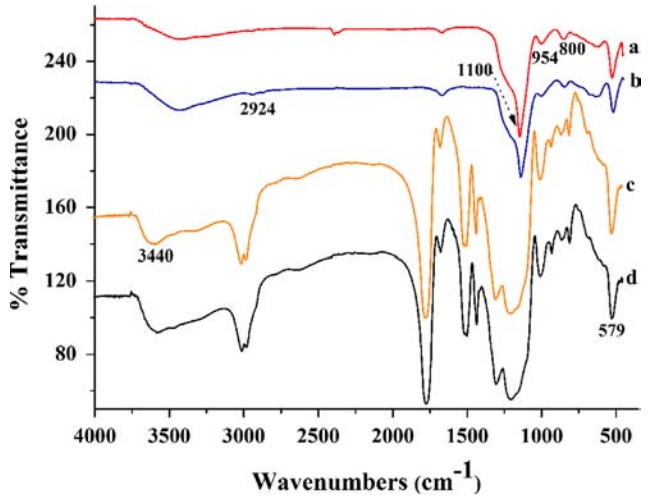

Fig. 4. FT-IR spectra of the Fe_3O_4@SiO_2 (curve a), Fe_3O_4@ SiO_2-MPS (curve b), MMIPs (curve c) and MNIPs (curve d).

3.3. Binding isotherms

The adsorption capacity of MMIPs for sildenafil was studied by static equilibrium adsorption. As shown in Fig. 5a, the amounts of sildenafil bound to MMIPs and MNIPs increase along with its initial concentration. At higher equilibrium concentration than 0.8 μmol mL^{-1}, adsorption of MMIPs became stable. However, the amount of sildenafil bound to the MMIPs is higher than that bound to the MNIPs, which illustrated the good specific binding of MMIPs for the template molecule and the success of the imprinting process.

In order to further study the binding properties of MMIPs and MNIPs, the obtained data were analyzed using the FI affinity distribution analysis model according to the following equation [28]:

$$\mathrm{Log}B = m\,\mathrm{log}F + \mathrm{log}a\,. \quad (1)$$

where B and F are the concentrations of bound and free analytes, respectively, m and a are the binding parameters. The values of m and a could be calculated from plotting Log B versus log F by a linear regression (Fig. 5b). The parameter m is the heterogeneity index, which varies from 0 to 1, with 1 being homogeneous and values approaching zero being increasingly heterogeneous. In addition, the number of binding sites per gram of material ($N_{kmin-kmax}$) and the weighted average affinity constant ($K_{kmin-kmax}$), were calculated using the following equations [28].

$$N_{kmin-kmax} = a(1-m^2)(K_{min}^{-m} - K_{max}^{-m}) \quad (2)$$

$$K_{kmin-kmax} = \left(\frac{m}{m-1}\right)\left(\frac{K_{min}^{1-m} - K_{max}^{1-m}}{K_{min}^{-m} - K_{max}^{-m}}\right) \quad (3)$$

The values for these parameters can be calculated from the experimental maximum (*Fmax*) and minimum (*Fmin*) free analyte concentrations and within the limits of *Kmin* and *Kmax* being equal to the corresponding reciprocal concentrations *Kmin*= *1/Fmax* and *Kmax*=*1/Fmin*.

The calculated fitting parameters (m, a, the number of binding sites, $N_{kmin-kmax}$, and the weighted average affinity constant, $K_{kmin-kmax}$) are summarized in Table 1. According to the m value, both of the materials contained a heterogeneous distribution of binding sites, and the MMIPs had a lower value of m than the MNIPs, which indicated a more heterogeneous structure. This was consistent with the previous works [17,21]. The binding sites of MMIPs for sildenafil measured were 1.457 μmol g^{-1} with an affinity constant of 16.657 L $mmol^{-1}$, while the binding sites 0.019 μmol g^{-1} of MNIPs were with an affinity constant of 1.579 L $mmol^{-1}$. The results indicated that MMIPs had higher binding affinity and selectivity to sildenafil than MNIPs.

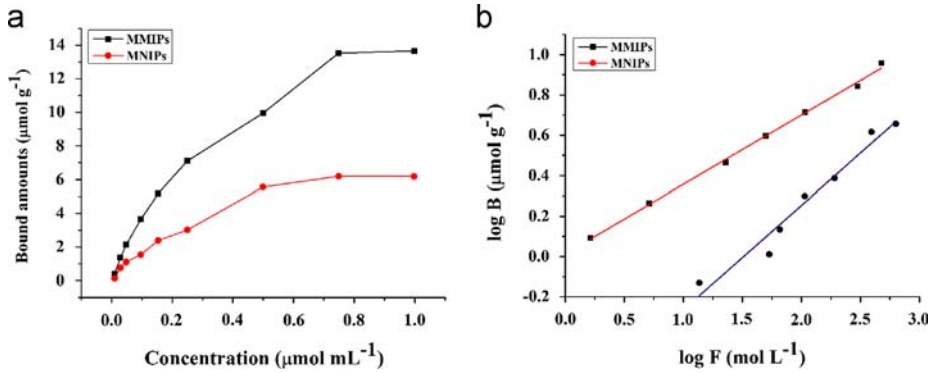

Fig. 5. (a) Sildenafil adsorption isotherms for MMIPs (■) and MNIPs (●) in the concentration ranging from 0.01 μmol mL^{-1} to 1 μmol $mL^{-1}$and (b) adsorption isotherms of sildenafil for MMIPs (■) and MNIPs (●) fitting to the Freundlich isotherm.

Table 1
Freundlich fitting parameters, number of binding sites ($N_{kmin-kmax}$) and weighted average affinity ($K_{kmin-kmax}$) for sildenafil on the MMIPs and MNIPs.[a]

Fitting parameters	MMIPs	MNIPs
$N_{kmin-kmax}$ (μmol g^{-1})	1.457 ± 0.003	0.019 ± 0.001
a [(μmol g^{-1}) ($mmol^{-1}$)^m]	1.101	0.137
$K_{kmin-kmax}$ (L $mmol^{-1}$)	16.657 ± 0.005	1.579 ± 0.002
m	0.396	0.597
r	0.9988	0.9717

[a] Data are shown as means ± S.D.

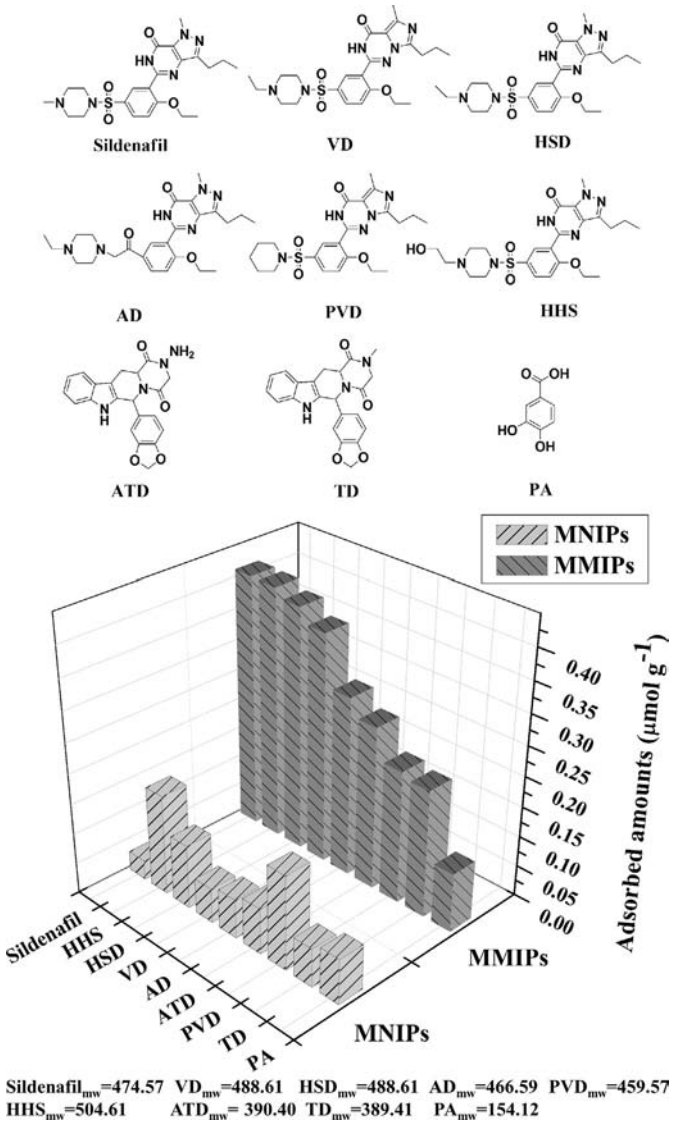

Fig. 6. Specific recognition capability of MMIPs and MNIPs to sildenafil, seven structurally similar compounds, and a reference compound. VD-vardenafil, HSD-homosidenafil, HHS-hydroxyhomosidenafil, PVD-pseudovardenafil, AD-acetildenafil, ATD-aminotadalafil, TD-tadalafil, and PA-protocatechuic acid.

3.4. Specificity evaluation of MMIPs

The molecular recognition of MMIPs could also been shown via the specificity test among different compounds. Fig. 6 shows adsorption amounts of sildenafil, HSD, HHS, PVD, ATD, AD, VD, TD, and PA with the MMIPs and MNIPs and the structures of them. Obviously, the adsorption amounts of the MMIPs for eight PDE-5 inhibitors are obtained at the range of 0.21–0.42 μmol g^{-1}, which is higher than the range from 0.06 to 0.17 μmol g^{-1} for MNIPs. It indicated that the MMIPs provided high selectivity to sildenafil and its structural analogs. The adsorbed amounts of MMIPs were related to the similarity between the target and template molecule. The interaction between polymer matrix and target was not only based on hydrogen bonding but also complement to target in size and shape. There was no obvious difference between the MMIPs and MNIPs to adsorb PA, which indicated that the adsorption for PA was non-specific.

Table 2
Recognition properties of MMIPs and MNIPs.[a]

	C_0 (μmol mL^{-1})		C_f (μmol mL^{-1})		K_d (mL g^{-1})		k	k'
	Sildenafil	PA[b]	Sildenafil	PA	K_{d1} Sildenafil	K_{d2} PA		
MMIPs	0.01	0.01	0.0024	0.0080	158.38	12.13	13.01	12.49
MNIPs	0.01	0.01	0.0082	0.0081	12.00	11.49	1.05	

[a] K_d, distribution coefficient. $K_d=(C_0-C_f)/C_f\times$ (solution volume [mL]/absorbent mass [g]), where C_0 and C_f represent the initial and final concentrations, respectively; k, selectivity coefficient. $k=K_{d1}/K_{d2}$; k', relative selectivity coefficient. $k_1'=k_{MMIP}/k_{MNIP}$.
[b] PA represents protocatechuic acid.

The competitive recognized coefficients of the MMIPs and MNIPs for the tested compounds were shown in Table 2. The distribution coefficient (K_d) is defined as the adsorbed-to-unabsorbed concentration ratio. The selectivity coefficient (k) is defined as the target-to-competitive molecule ratio of the K_d values. The relative selectivity coefficient (k') is defined as the target-to-competitive molecule ratio of the k values. K_d suggests the adsorption capacity. The larger the value of K_d is, the stronger the adsorption capability of a substance will be. The parameter k indicates the selectivity between two substances, whereas k' reflects the selective difference between MMIPs and MNIPs. The larger the value of k' is, the greater the selectivity of molecular imprinting will be. As seen in Table 2, the MMIPs demonstrated high adsorption selectivity for sildenafil, and there was no obvious difference in binding sildenafil and PA for MNIPs. The selectivity of MMIPs ($k=13.01$) was almost 12.5 times ($k'=12.49$) greater than that of MNIPs, which indicated that the imprinting process significantly improved adsorption selectivity to the imprinted template and no specific site was suited for the compound with significantly different structures.

3.5. Extraction procedure

It is necessary to optimize conditions in order to extract as much analytes as possible. Acetonitrile was used as extraction solvent with the MMIPs. Different amounts of MMIPs ranging from 5 to 100 mg in 1 mL extraction solvent were applied to the eight PDE-5 inhibitors (0.5 nmol mL^{-1} for each). The results in Fig. 7a show that 50 mg were enough for the extraction; further increasing amounts of MMIPs had no effect for increasing the recoveries of the targets.

Extraction times (i.e., 5, 10, 15, 20, 30, and 40 min) were conducted in 1 mL mixed solution of eight PDE-5 inhibitors (0.5 nmol mL^{-1} for each). Fig. 7b shows that the MMIPs reach adsorption equilibrium at approximately 20 min for the eight PDE-5 inhibitors. The bounding recoveries of the eight targets were ranged from 44.9% to 87.3%. Therefore, 20 min extraction time was selected in the following experiments.

Different time intervals (i.e., 5, 10, 15, 20, 30, and 40 min) were evaluated to obtain desorption time of the eight target analytes.

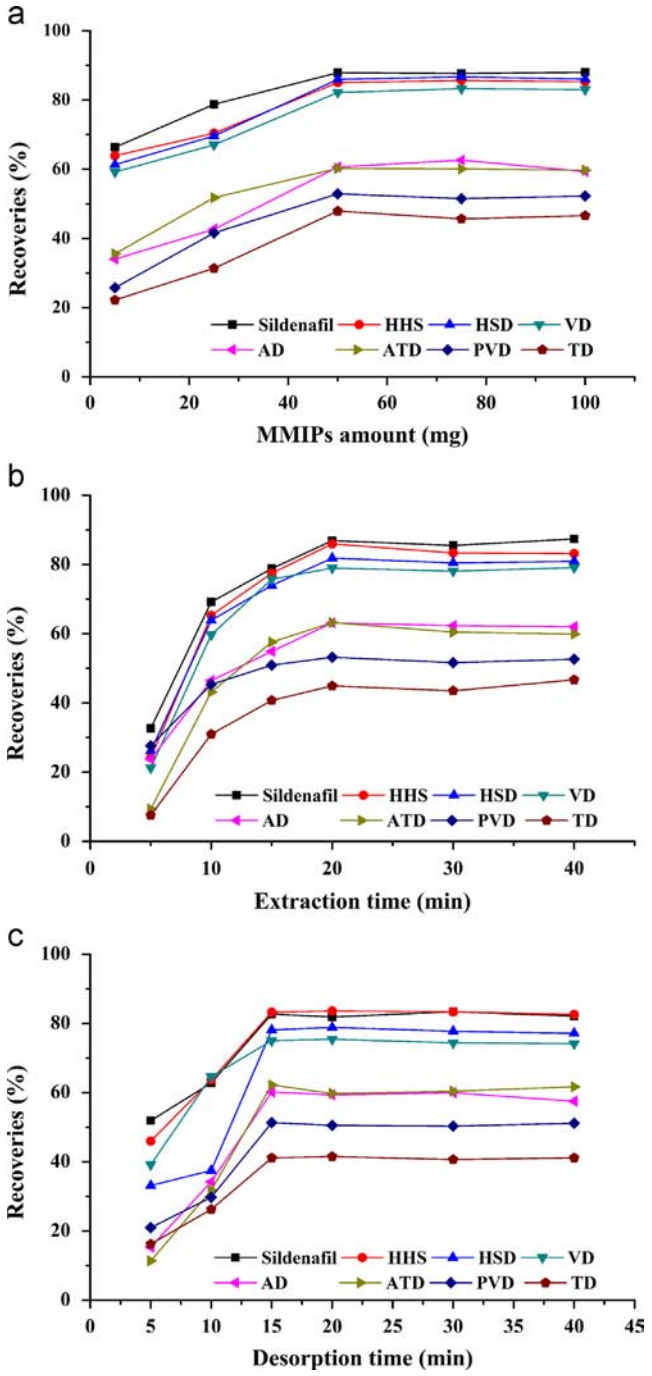

Fig. 7. The effects of MMIPs amount (a), extraction time (b), and desorption time (c) on the recoveries of the PDE-5 inhibitors.

Fig. 7c illustrates that 15 min is sufficient to accomplish desorption period, and the MMIPs could be isolated in a short time (approximately 1 min) by an external magnetic field. Therefore, desorption time was set at 15 min.

3.6. Application of the MMIPs in real samples

A series of sildenafil, HSD, HHS, PVD, ATD, AD, VD, and TD standard solutions were investigated for the linearity of the MMIPs extraction coupled with the HPLC method. Good linearity was achieved in the range of 0.1–2 nmol mL^{-1} for sildenafil, HSD, HHS, PVD, VD, and TD, and 0.15–2 nmol mL^{-1} and 0.25–2 nmol mL^{-1} were obtained for ATD and AD, respectively. The correlation coefficient was at the range of 0.9953–0.9992. The limits of detection (LOD) for eight PDE-5 inhibitors were in the range of 7.79–23.33 ng mL^{-1}, and limits of quantification (LOQ) were in the range of 27.26–81.66 ng mL^{-1}. The standard addition method was used to evaluate the repeatability, accuracy and recovery of the MMIP-HPLC extraction process. The medicine extracts were spiked with the eight PDE-5 inhibitors at three concentration levels. The results are summarized in Table 3. The recoveries of the spiked samples for the eight PDE-5 inhibitors range from 48.5% to 94.8% with the RSD% values ranging from 1.93% to 9.86%. The recoveries of TD and PVD were less than 60% because of a little different structure with sildenafil template. The degree of molecular analogy to the template was relative to the extraction yields of MMIPs. The comparison of some methods used for determination of PDE-5 inhibitors was listed in Table 4. These results demonstrated that the MMIPs had high selectivity and enrichment ability for the analysis of six structurally similar PDE-5 inhibitors in complex medicine matrix.

The present study aimed to provide a simple, selective, and practical process by using MMIPs, which can be applied in the determination of analytes from complicated samples. The chromatogram of direct injection of the two spiked (5 nmol g^{-1}) medicine extracts Guilingji (sample 1) and Shenrong capsule (sample 2), the spiked medicine extract samples extracted by MMIPs and MNIPs (5 nmol g^{-1}), and the mixed standard solution was shown in Fig. 8. The eight PDE-5 inhibitors could not be directly determined from the complicated medicine samples through HPLC without enrichment (Fig. 8a). No selective peak was observed in Fig. 8b in the analysis of the solutions extracted by MNIPs, and the selectivity for eight PDE-5 inhibitors using the proposed MMIP extraction method was enhanced successfully (Fig. 8c). The content of ATD in sample 1 was found to be 6.51 μg g^{-1}. The baseline obtained for the analysis of extracts by MMIPs was as clean as that shown in Fig. 8d for the standard solution. The results indicated that the prepared MMIPs can be used in the field of selective determination of multi-target analytes in complicated samples.

Table 3
Accuracy of the method for samples solution spiked at different concentrations ($n=3$).

	Sample 1						Sample 2					
	5 nmol g^{-1}		6.5 nmol g^{-1}		8 nmol g^{-1}		5 nmol g^{-1}		6.5 nmol g^{-1}		8 nmol g^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
AD	61.1	6.18	65.6	2.70	65.7	2.19	60.0	6.69	64.2	3.53	65.4	3.69
ATD	62.3	4.45	64.2	3.48	68.1	4.48	60.2	5.27	65.6	5.06	67.5	3.19
TD	49.5	6.93	50.9	7.47	53.2	3.99	48.5	6.00	50.3	9.86	53.0	3.42
HHS	84.7	6.49	88.1	4.54	85.2	3.93	77.8	2.86	81.3	4.40	82.4	5.61
Sildenafil	93.4	1.93	94.3	3.79	92.6	4.65	92.3	6.01	94.7	5.89	91.7	2.43
VD	74.4	3.76	77.2	5.42	80.0	5.10	72.9	3.84	74.9	6.86	78.3	3.04
HSD	72.9	7.17	78.2	3.61	77.9	4.17	69.9	5.68	79.7	8.51	79.1	5.08
PVD	54.7	4.21	57.0	3.52	57.4	7.43	57.0	2.11	54.8	3.52	54.7	5.20

Table 4
Comparison of some methods used for determination of PDE-5 inhibitors.

No.	Matrix	Target compounds	Extraction method	Detection	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Recovery (%)	Ref.
1	Herbal medicine samples	Sildenafil Vardenafil	MMIPs	HPLC–MS	3.66 4.15	–	89.7–91.7 70.9–78.2	[23]
2	Herbal medicine samples	Vardenafil	Immumoassay	UV	5	–	76.0–116.0	[29]
3	Cosmetic creams	Sildenafil Vardenafil	Ultrasonic treatment	LC–ESI-MS	2.0, 2.7 ng/g	6.6, 8.9 ng/g	94.8–95.7 92.8–96.9	[30]
4	Dietary supplements	Sildenafil Vardenafil Homosildenafil	Shaking extraction	GC–MS	200	–	–	[31]
5	Human serum	Sildenafil	Ionic liquid	CE-MS	14	–	97.6–100.5	[15]
6	Herbal medicine samples	Sildenafil Vardenafil Homosidenafil Hydroxyhomosidenafil Acetildenafil Aminotadalafil	MMIPs	HPLC-DAD	9.49 9.77 10.2 10.1 23.3 11.7	31.3 32.3 35.9 35.5 81.7 41	60.0–94.7	This one

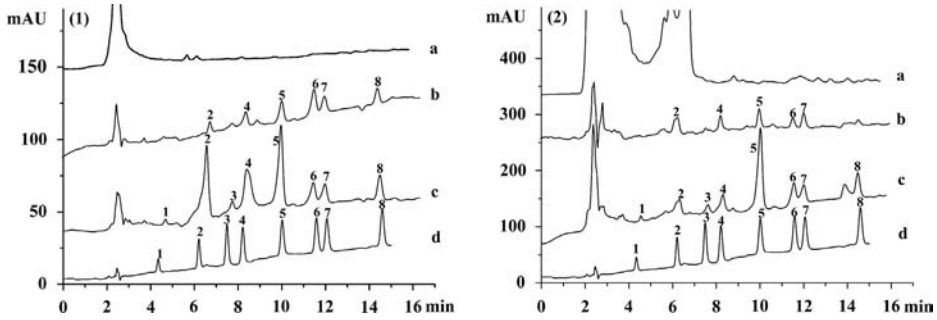

Fig. 8. Chromatograms of two herb medicine samples: (a) eight PDE-5 inhibitors spiked extraction solution of sample. (b) Spiked solution extracted with MNIPs. (c) Spiked solution extracted with MMIPs. (d) Eight PDE-5 inhibitors mixed standard solution; 1-AD, 2-ATD, 3-TD, 4-HHS, 5-sildenafil, 6-VD, 7-HSD, and 8-PVD.

4. Conclusions

The MMIPs were prepared as selective extraction sorbents for the analysis of PDE-5 inhibitor adulterants in complicated medicine matrix. The obtained MMIPs were characterized via TEM, FT-IR, XRD, and VSM. Investigation of selectivity recognition properties showed high adsorption capacity and selectivity of the MMIPs to template molecule and structure similarly compounds. The extraction procedure took a short time to reach adsorption and desorption equilibrium and the MMIPs were easily collected using an external magnetic field. The proposed method was successfully developed toward six PDE-5 inhibitors: sildenafil, vardenafil, homosidenafil, hydroxyhomosidenafil, acetildenafil, and aminotadalafil. It was the first time a group of PDE-inhibitors were tested for the MMIPs and the use of MNIPs allowed discrimination of nonselective binding events. Good recoveries and low detection limits were obtained.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.009.

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