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Imprinted functionalized silica sol–gel for solid-phase extraction of triazolamin

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ABSTRACT

A triazolam-imprinted silica microsphere was prepared by combining a surface molecular-imprinting technique with the sol–gel process. The results illustrate that the triazolam-imprinted silica microspheres provided using γ-aminopropyltriethoxysilane and phenyltrimethoxysilane as monomers exhibited higher selectivity than those provided from γ-aminopropyltriethoxysilane and methyltriethoxysilane. In addition, the optimum affinity occurred when the molar ratio of γ-aminopropyltriethoxysilane, phenyltrimethoxysilane, and the template molecule was 4.2:4.7:0.6. Retention factor (k) and imprinting factor (IF) of triazolam on the imprinted and non-imprinted silica microsphere columns were characterized using high performance liquid chromatography (HPLC) with different mobile phases including methanol, acetonitrile, and water solutions. The molecular selectivity of the imprinted silica microspheres was also evaluated for triazolam and its analogue compounds in various mobile phases. The better results indicated that k and IF of triazolam on the imprinted silica microsphere column were 2.1 and 35, respectively, when using methanol/water (1/1, v/v) as the mobile phase. Finally, the imprinted silica was applied as a sorbent in solid-phase extraction (SPE), to selectively extract triazolam and its metabolite, α-hydroxytriazolam, from human urine samples. The limits of detection (LOD) for triazolam and α-hydroxytriazolam in urine samples were 30 ± 0.21 ng mL^{-1} and 33 ± 0.26 ng mL^{-1}, respectively.

1. Introduction

A short-acting benzodiazepine used in the treatment of insomnia, triazolam has been used as an anti-anxiety agent and a muscle relaxant. Drug abusers take triazolam illicitly, sometimes together with other drugs and alcohol, either to calm themselves down or to amplify a drug-induced high. When consumed, triazolam is metabolized in the liver, mainly producing α-hydroxytriazolam [1]. Triazolam is frequently encountered in clinical and forensic toxicology. Thus, it is necessary to develop a sensitive and reproducible method to determine triazolam and its metabolite at the low levels encountered in biological samples, such as urine or blood. Several methods have been reported for the determination of triazolam in biological samples using gas chromatography (GC) [2], GC–mass spectrometry (GC–MS) [3], high-performance liquid chromatography (HPLC) [4], HPLC–MS [5,6] and LC–MS/MS [7]. Prior to the employment of these instrumental protocols, drugs were commonly extracted and cleaned up by appropriate procedures to remove possible interfering contents of the matrices. Solid-phase extraction (SPE) is a well-established method for sample cleanup and preconcentration and molecularly imprinted polymers (MIPs) appear to be well-suited for this application.

MIPs are stable synthetic polymers possessing selective molecular recognition sites, which are obtained by using large amounts of cross-linking monomers in the presence of a template molecule. Once the template molecule is removed, specific sites left behind are able to selectively rebind to the template and structurally similar analytes. Furthermore, MIPs are usually reusable, have a low preparation cost, exhibit high mechanical and chemical stability, and can be used in an extensive range of operating conditions [8]. For these reasons, MIPs can be used as powerful sorbents for the extraction of target analytes from complex matrices [9–12].

Various imprinted materials have been made, but have demonstrated limitations including incomplete template removal, minimal binding capacity, slow mass transfer rate, and irregular material shapes. The use of MIPs as the stationary phase in HPLC has generated important problems, such as high backpressure and low mass transfer kinetics, resulting in broad and tailed peaks [13].

Surface molecularly imprinted polymers possess high selectivity and high transfer rates. By focusing on just the surface of a support or a thin layer on a surface, fast uptake/release of the target analyte can be achieved. The sol–gel method allows synthesis of MIPs with uniform porosity and surface area, plus selectivity and diffusion rates which are equivalent to or better than those of

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acrylic polymer-based MIPs [14], which require special experimental conditions for their preparation [15].

Surface imprinting techniques combined with the sol–gel process for MIP-SPE sorbent preparation have been extensively developed and applied [16–20]. Recently, several research groups have begun to synthesize molecularly imprinted sol–gel material by combining a surface molecular-imprinting technique with a sol–gel process. Marx et al. have created specific cavities in thin sol–gel-derived films by utilizing (R)- or (S)-propranolol, parathion, or paraoxon as templates [21–23]. Silica is an ideal support for organic groups because it is a non-swelling inorganic material, stable under acidic conditions and, additionally, it has high mass exchange characteristics and very high thermal resistance [24]. Yan and co-workers have prepared surface cadmium (II)-imprinted sol–gel materials via couple grafting of the polymer and ionic imprinting on the surfaces of silica particles (80–120 mesh) [25].

In this study, the synthesis of a molecular imprinting polymer of agglomerate nanoparticles grafted on the surface of silica gel microspheres (MIP-SiO_2) specific for triazolam, and its application to urine, are described. The results obtained are the basis for further studies on the application of MIP-SiO_2 as a sorbent for HPLC columns. The formation of specific and selective binding sites for triazolam in a sol–gel matrix was examined. Functional silanes, containing phenyl and primary amine groups, were chosen due to their ability to interact noncovalently with triazolam within the imprinting polymer and to provide the chemical anchors for rebinding. The role of the functional monomers and their requirement for providing several points of noncovalent interaction were examined. A molecularly imprinted solid-phase extraction (MISPE) protocol for triazolam has been developed and tested for triazolam and its analogues (Fig. 1).

2. Experimental

2.1. Materials

The self-made silica (diameter about 10 μm) was used as the support medium to prepare the surface triazolam-imprinted sorbent. Tetraethoxysilane (99%, TEOS), phenyltrimethoxysilane (98%, PTMOS), 3-aminopropyltriethoxysilane (97%, APTEOS), methyltriethoxysilane (97%, MTEOS), methanesulfonic acid (98%), methanol (HPLC grade), tetramethyl ethylenediamine (HPLC grade), and acetic acid (HPLC grade) were purchased from Alfa Aesar Chemicals Reagent Factory (Ward Hill, MA, USA). Triazolam, α-hydroxytriazolam, barbital, phenobarbital, and pentobarbital were sourced from Guangzhou Forensic Science Institute (Guangzhou, China). Estazolam and the other benzodiazepine analogues were generously provided by Changzhou No. 4 Pharmaceutical Factory (Jiangsu, China). The mobile phase used for the HPLC experiments was a mixture of methanol, water, tetramethyl ethylenediamine and acetic acid (700:300:4.4:3.2), and was filtered through a 0.45-μm filter prior to use. All reagents were analytical grade or better.

2.2. Instrumentation

The chromatographic system employed consisted of a Shimadzu (Kyoto, Japan) LC-10AD pump, and a Shimadzu SPD-10A UV–VIS detector (Detection wavelength was 254 nm); the sample loop volume was 20 μL in HPLC. All separations were undertaken on an analytical reversed-phase column (Symmetry-C_{18}, 5 μm, 4.6 mm i.d. × 15 cm long), purchased from Dikma Technologies (Beijing, China), at a flow rate of 1.0 mL min^{-1} under isocratic conditions at room temperature. The SEM micrographs were obtained with met-

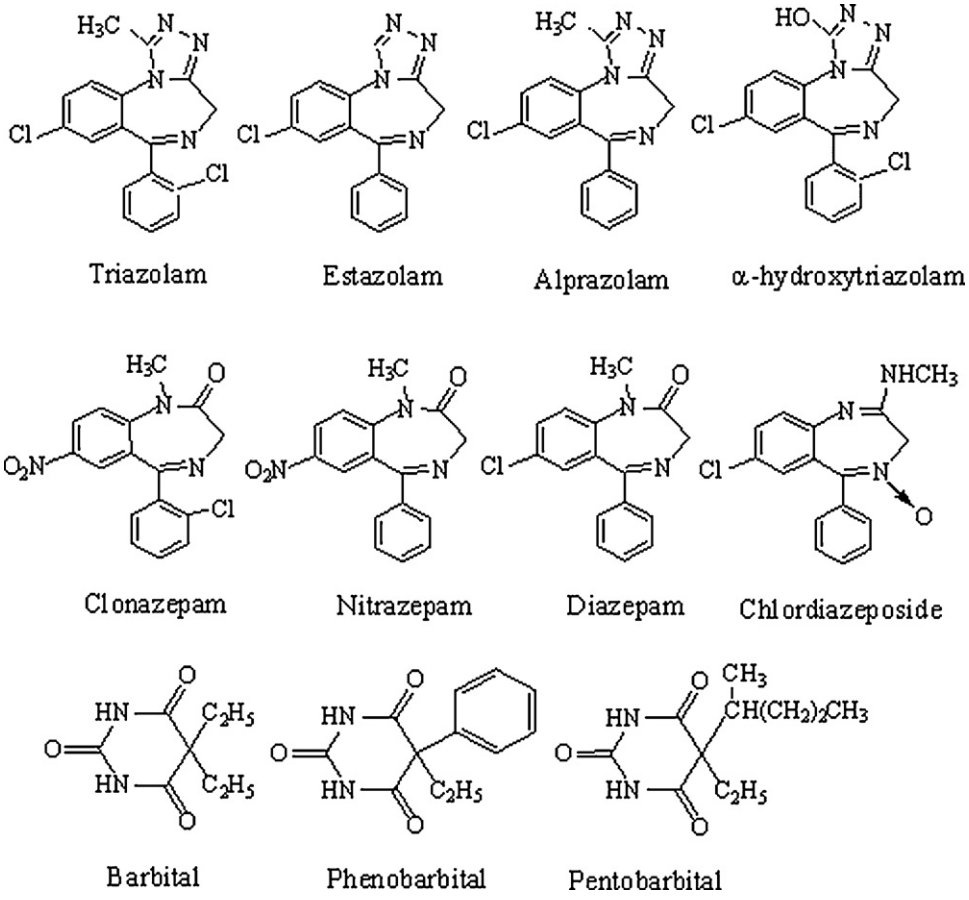

Fig. 1. The structures of triazolam, barbital, and their analogues.

allization of the silica samples on a Hitachi (Kyoto, Japan) S-520 scanning electron microscope. FT-IR spectra were recorded with KBr pellets in transmission mode, using a PE Spectrum One FT-IR Spectrometer produced by PerkinElmer Company (Foster City, CA, USA). The solid-phase extraction installation was obtained from SUPELCO Company (Bellefonte City, PA, USA). The C_{18} SPE cartridge (containing 0.1 g sorbent) was obtained from the Borei Bonding Company (Tianjin, China).

2.3. Procedures for the preparation of triazolam-imprinted polymers on the surface of silica

The surface of silica was activated according to the method previously described by Yan and co-workers [26].

8 g of silica were mixed with 60 mL of 33% (w/w) methanesulfonic acid and refluxed for 8 h at 110 °C while stirring. The solid product was recovered by filtration, washed with double-distilled, deionized water to neutralize, and then dried under vacuum at 70 °C for 8 h.

To prepare the different triazolam-imprinted silica samples, triazolam was dissolved in 15 mL of methanol while stirring, then APTEOS and PTMOS (or MTEOS) were added into the mixture (see Table 1 for the different concentrations used). The fixed amount of TEOS was added after stirring for 30 min. The solution was stirred for another 20 min, after which 1 g of activated silica and 1 mL of 0.012 mol L^{-1} HCl were added. The mixture began to co-hydrolyze and co-condense after stirring for an additional hour, then incubating for 24 h at 30 °C. The product was filtered and dried in a vacuum oven at 100 °C for 12 h. At this point, the activated silica surface was grafted with the molecularly imprinting polymer. To remove the template, the triazolam-imprinted silica was Soxhlet-extracted with a solution of acetic acid in methanol (10% by volume) for 24 h. Continuous washing with methanol removed any residual acetic acid. The imprinted silica (MIP-SiO_2) was then dried under vacuum at 80 °C until its weight was constant.

Two reference non-imprinted silica (NIP-SiO_2) were prepared in the same way, but without the template (see Table 1).

2.4. Evaluation of the performance of the imprinted silica microspheres

2.4.1. Static binding test

20 mg of imprinted or non-imprinted silica were mixed with 2.0 mL of solution (methanol/water, 1/9, v/v) containing 80 mg L^{-1} of triazolam. The mixtures were incubated for 6 h, with continuous shaking in a horizontal shaker at room temperature. After incubating, the mixtures were filtered through a 0.45 μm filter prior to determining the amount of unextracted triazolam. The Q (mg g^{-1}) is the ratio of the amount of the extracted triazolam (mg) and the imprinted or non-imprinted silica (g) using in the test.

2.4.2. Liquid chromatography

The MIP-SiO_2 or NIP-SiO_2 was slurry-packed into a stainless steel column 15 mm × 4.6 mm i.d. Different mobile phases were investigated, including mixtures of methanol/water, acetonitrile/water, methanol/acetonitrile/water, and methanol/water containing tetramethyl ethylenediamine/acetic acid buffer. The mixtures had ratios between 100:0 and 50:50 (v/v). Analyses were performed at a flow rate of 0.5 mL min^{-1} in the isocratic mode using a detection wavelength of 254 nm. A sample volume of 10 μL was injected. The retention factor $k = (t - t_0)/t_0$, where t is the retention time of a given analyte and t_0 is the retention time of the void volume marker ($NaNO_2$), and the imprinting factor $IF = k_{MIP}/k_{NIP}$, where k_{MIP} is the retention factor of the MIP and k_{NIP} is the retention factor of the NIP, were calculated.

2.4.3. Solid-phase extraction

A polyethylene frit was inserted into an empty 1 mL polypropylene SPE cartridge. The cartridge was connected to a vacuum manifold and 100 mg of the imprinted or non-imprinted silica were packed into the cartridges before insertion of another frit on top of the sorbent bed. 5 × 1 mL methanol and 3 × 1 mL loading solvent were successively passed through the imprinted or non-imprinted silica cartridge to equilibrate them. Then the standard solution containing the analyte in methanol/water (1/9; v/v) was loaded into the imprinted silica cartridge at a flow rate of 0.5 mL min^{-1}. After the sample loading, air was passed through the cartridge for 10 min, and the cartridge was washed with a corresponding solvent. Finally, the elution was performed by passing 1 mL of eluent. The collected elute was completely evaporated under vacuum and redissolved in 200 μL of methanol prior to analysis by HPLC. Each extraction was repeated three times.

2.5. MISPE in urine samples

2.5.1. Preparation of urine samples

The urine samples were prepared according to the method previously described by De Leenheer and co-workers [27]. 4 mL of urine were added to a 50-mL amber centrifuge tube. The urine was buffered with 8 mL 0.2 M sodium acetate buffer (pH 4.5) and 11,630 u of β-glucuronidase were added. The tube was shaken vigorously and incubated at 56 °C for 2 h. After centrifugation at 1500 rpm for 10 min, the supernatant was transferred to a 50-mL amber centrifuge tube. 40 μL of 1 M sodium hydroxide and 8 mL phosphate buffer (pH 6.8, containing 2 mL methanol) were added to the tube prior to solid-phase extraction.

2.5.2. MISPE of urine samples

The MISPE cartridge was preconditioned with 5 × 1 mL of methanol followed by 3 × 1 mL methanol/pH 6.8 phosphate buffer (1/9, v/v). The 5 × 1 mL prepared urine samples were loaded into the MISPE columns at a flow rate of 0.5 mL min^{-1}. After the sample loading, air was passed through the cartridge for 10 min. The MISPE column was then washed with 2 mL of deionized water and 3 × 1 mL

Table 1
Molar ratios of different functional monomers, template, and cross-linking reagent used in the synthesis of imprinted silica microspheres.

	Triazolam (mmol)	APTEOS (mmol)	PTMOS (mmol)	MTEOS (mmol)	TEOS (mmol)	Methanol (mL)
MIP1	1.8	4.2	4.7	0	18	15
MIP2	1.2	4.2	4.7	0	18	15
MIP3	1.0	4.2	4.7	0	18	15
MIP4	0.8	4.2	4.7	0	18	15
MIP5	0.6	4.2	4.7	0	18	15
MIP6	0.5	4.2	4.7	0	18	15
MIP7	0.6	4.2	0	4.7	18	15
NIP1-6	0	4.2	4.7	0	18	15
NIP7	0	4.2	0	4.7	18	15

methanol/water (1/9, v/v), and eluted with 1 mL methanol. 200 ng of the diazepam internal standard were added before the collected elute was completely evaporated under vacuum, and the residues were redissolved in 200 μL of methanol prior to analysis by HPLC. Each extraction was repeated three times.

In this experiment, the urine samples were spiked consecutively with 0.2, 0.4, 1.0, 2.0, and 4.0 μg mL^{-1} each of triazolam and α-hydroxytriazolam.

3. Results and discussion

3.1. Selection of the functional monomers

A basic requirement for successful molecular imprinting is the selection of suitable functional silane monomers, which can create the molecular recognition site by interacting with the template molecule in a noncovalent mode, and can leave interacting chemical functional groups situated within the cavity in an optimal position for rebinding after the polymerization. PTMOS contains a phenyl group, which may interact with triazolam via a π–π interaction. To a certain extent, the primary amine of APTEOS is capable of establishing hydrogen bonds with the electron-acceptor group (e.g., C–N=) of the triazolam. TEOS provides the hydrophobic ether-like Si–O–Si bond. The imprinted silica was synthesized over 24 h because of the slow hydrolysis of TEOS. Static binding of triazolam was measured for seven possible compositions for the imprinted and the non-imprinted silica (Fig. 2). The data clearly indicate that the binding capacity of MIP5 was the highest among the seven imprinted silicas. When MIP7 was used, low specific binding capacity was observed. In this case, triazolam seems to be bound to the imprinted silica through the weak H-bonds with the remaining free silanol groups. Since the cavity is poorly defined in terms of chemical functional groups, the three-dimensional information is not sufficient for specific binding. When PTMOS was substituted for MTEOS, some degree of specific binding was observed. This may be due to the strong π–π interaction between the phenyl groups of PTMOS and triazolam. It is possible that, during the polymerization, triazolam is associated with a functional monomer in a multicomponent complex like it is suggested in Fig. 3. It is assumed that the chlorophenyl group is accommodated into the pocket created by the surface imprinting through π–π interaction with the phenyl group in the imprinted silica, which participates in the recognition mechanism of the triazolam. The results indicate that the adsorption of triazolam onto the imprinted silica was a combination of π–π interaction, hydrophobic interaction, and hydrogen bonding. For this reason, MIP5 was selected for the subsequent experiments.

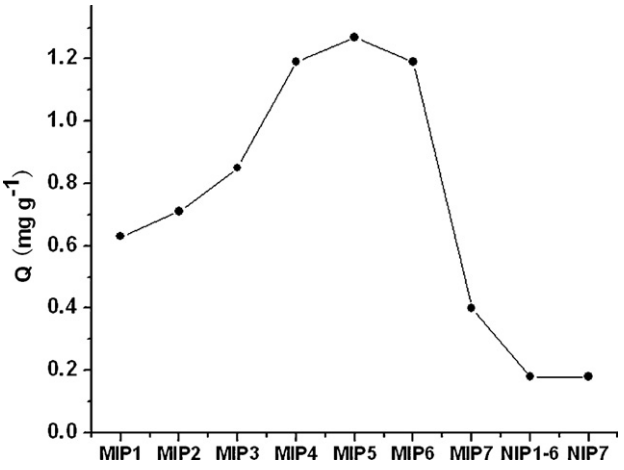

Fig. 2. The effect of functional monomers on the binding capacity of imprinted silica microspheres (synthesized according to Table 1).

3.2. Chemical and structural characterization of the imprinted silica

Fig. 4 shows the IR absorption spectra for the activated silica and the imprinted silica. The observed features at 1050 and 968 cm^{-1} indicate Si–O–Si and Si–O–H stretching vibrations, respectively. O–H vibration is reflected at 3626 cm^{-1}, and bands at 806 and 479 cm^{-1} result from Si–O vibrations. Compared to activated silica, the distinctive absorption spectra for the imprinted silica is characterized by the features of unsaturated C–H bond at 3056 cm^{-1}, saturated C–H bond at 2945 cm^{-1}, and the single substituent on

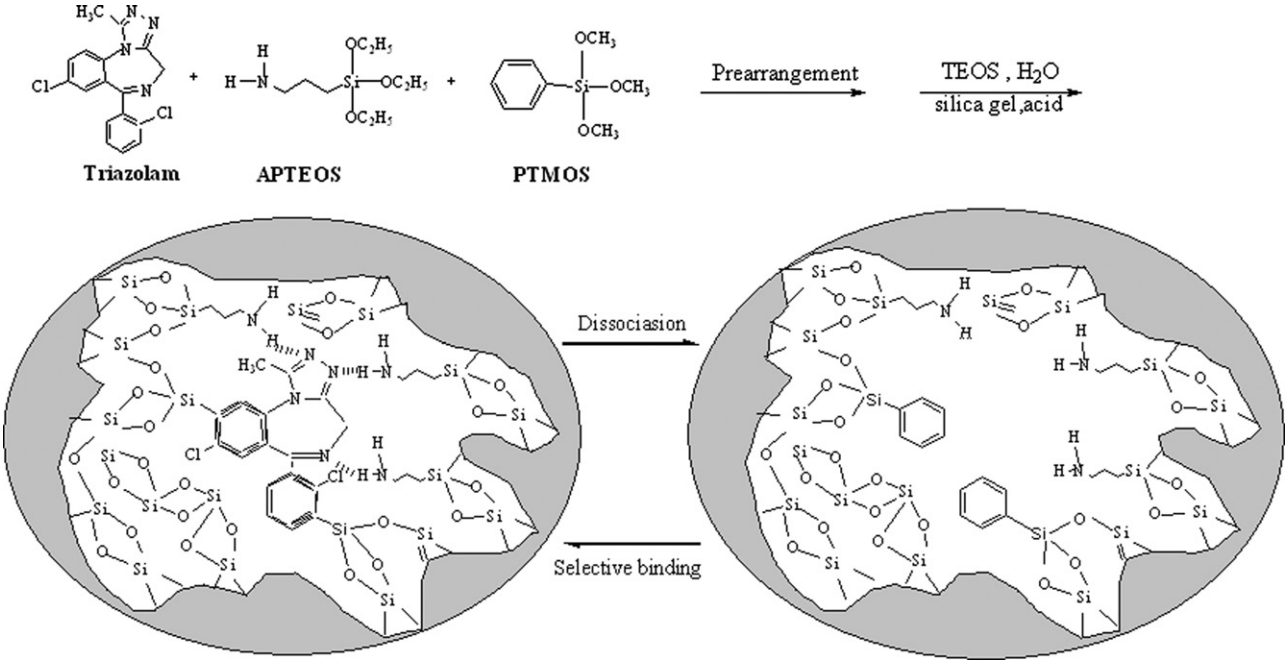

Fig. 3. Scheme for the synthesis of imprinted silica microspheres.

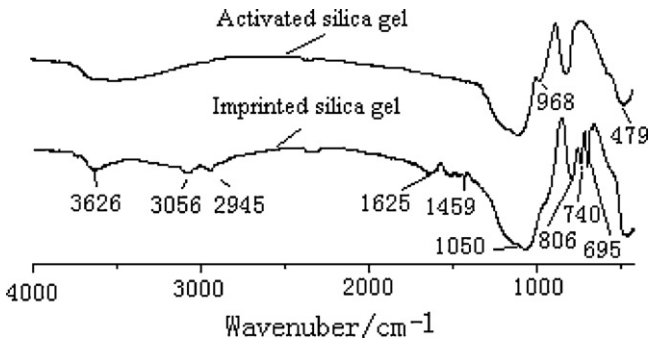

Fig. 4. FT-IR spectra of activated and imprinted silicas.

the benzene ring at 740 and 695 cm^{-1}. The N–H distortion vibration was inconspicuous because the framework vibration features of benzene were reflected from 1459 to 1625 cm^{-1} [28].

Scanning electron microscopy (SEM) photographs for both the activated and the imprinted silica are provided in Fig. 5. The separate imprinted silica microspheres can be observed in Fig. 5(b). The magnified image of the imprinted silica (Fig. 5(d)) verified the formation of agglomerates of nanoparticles with multi-porous surfaces after the hydrolytic condensation of the siloxane. These agglomerates are not seen in the space among the microspheres (see Fig. 5(b)). The surface of the activated silica (Fig. 5(a and c)) was much smoother than that of the imprinted silica microspheres.

3.3. Chromatographic evaluation

3.3.1. Effect of mobile phases

To evaluate the imprinting effect of the imprinted silica and to identify the influence of the mobile phase composition on the retention of triazolam, HPLC analyses were performed. The influence of the concentration of water in the mobile phase of the *k* and IF values of triazolam was investigated. As shown in Fig. 6, *k* increased as the volume ratio of water in the mobile phase increased. Triazolam was not eluted from the column when the mobile phase contained more than 50% water in mixtures with methanol. The increase in *k* of triazolam obtained with mixtures containing methanol was much higher than that obtained with mixtures containing acetonitrile.

The retention factors of triazolam on the imprinted and non-imprinted silica columns were different, and the IFs were observed for various mobile phases. The IF of triazolam for the imprinted silica column increased with increasing water concentration in the mobile phase, and was highest for methanol containing 50% water (IF = 35) and acetonitrile containing 50% water (IF = 1.7). The H-bonding interaction between triazolam and the interaction sites of

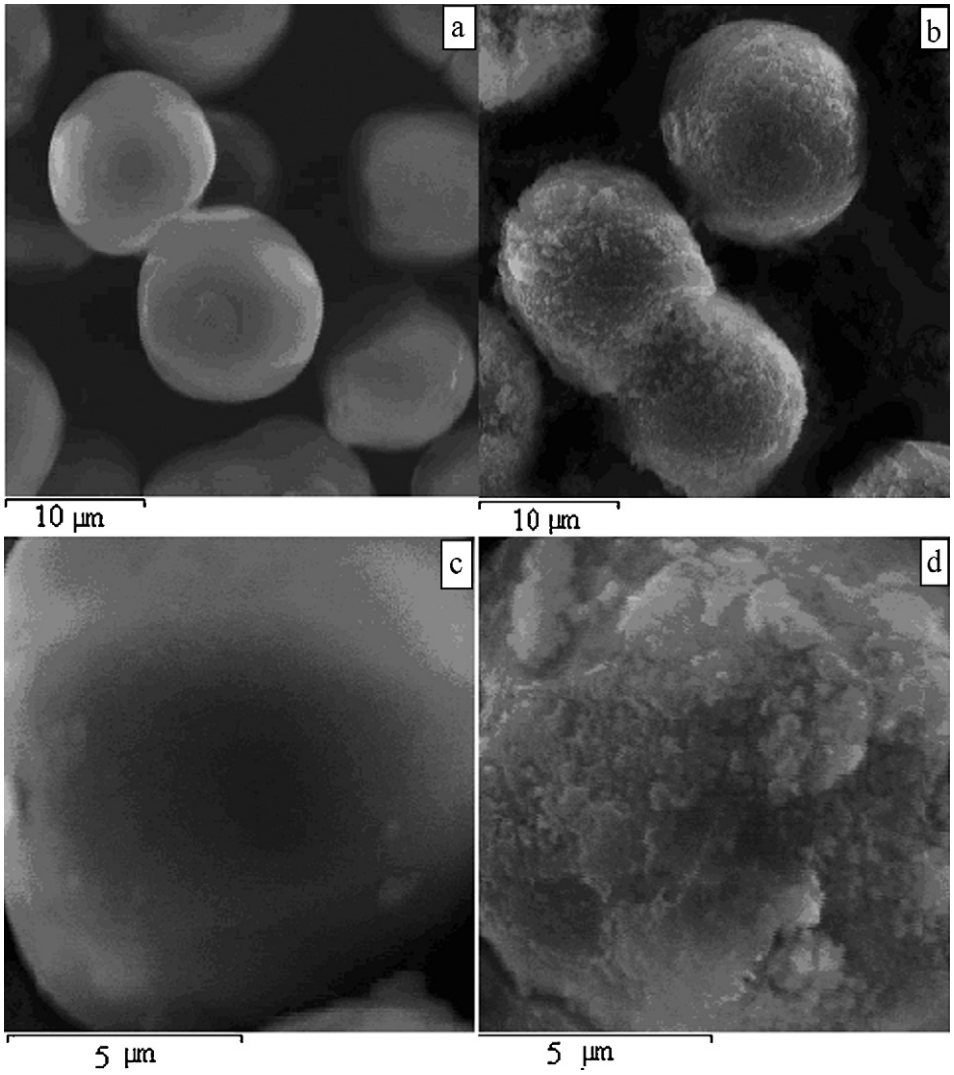

Fig. 5. SEM images of activated silica microspheres (magnification: (a) 3000×, (b) 10,000×), and imprinted silica microspheres (magnification: (c) 3000×, (d) 10,000×).

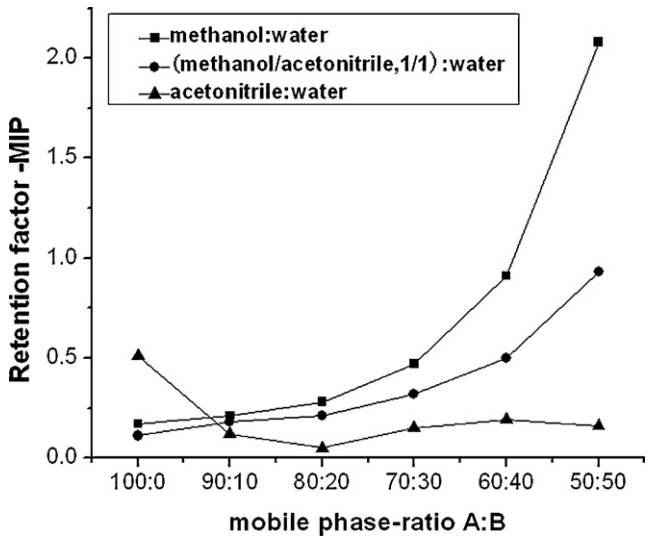

Fig. 6. Retention factor (k) of triazolam on the imprinted silica column using different mobile phases. Conditions: isocratic elution at a flow rate of 0.5 mL min^{-1}; injection volume = 10 μL. Column dimensions = 15 mm × 4.6 mm, detection wavelength = 254 nm, void time (t_0) marker $NaNO_2$; $k = (t - t_0)/t_0$.

the imprinted silica decreased in aqueous mobile phases [29], but the imprinted silica had a higher affinity for triazolam than did the non-imprinted silica. The results may arise from the strong π–π bond interaction between the imprinted silica and the template. This interaction is more dominant than hydrogen bonding; thus its contribution is larger. Furthermore, the imprinted complex defines the size and orientation of the chemically functionalized cavity for binding that is not present in the non-imprinted silica.

The optimum mobile phase for the extraction of triazolam was methanol/water (1/1, v/v). The effect of pH on the adsorption of triazolam was tested by using methanol/water (1/1, v/v) containing tetramethyl ethylenediamine/acetic acid buffer as the mobile phase under different pH conditions. The k and IF of triazolam on the imprinted silica column increased as the pH of the mobile phase increased, from 0.6 and 7 at pH 3 to 1.4 and 24 at pH 7. The extraction efficiency was minimal for an acidic sample as the analyte itself was basic; therefore the extraction efficiency of the imprinted silica for a basic sample was higher. A similar effect was observed in our previous study [30].

3.3.2. Selectivity of the imprinted silica in HPLC column

Selective recognition studies were performed with triazolam and structurally related compounds. Table 2 summarizes the data for the separation factors (a) of the target molecules on the imprinted silica column using various ratios of methanol to water as the mobile phase. The selective factors of the benzodiazepine molecules increased as the ratio of water to methanol increased from 0/10 to 3/7. This may result from the imprinting effect and the difference in the molecular interactions.

3.4. Molecularly imprinted solid-phase extraction

3.4.1. Binding capacity of the imprinted silica microsphere

10 mL of methanol/water (1/9, v/v) solution with triazolam, α-hydroxytriazolam, alprazolam, nitrazepam, or phenobarbital at a concentration of 2 mg L^{-1}, were passed through the imprinted and non-imprinted silica cartridges. The specificity of the imprinted silica compared with that of the non-imprinted silica is provided in Table 3. The binding capacities of triazolam, α-hydroxytriazolam, alprazolam, nitrazepam, and phenobarbital on the imprinted silica were 5.6, 7.8, 1.3, 2.3, and 1.2 times larger than those on the non-imprinted silica, respectively. The results indicate that the imprinted silica had higher selectivity than the non-imprinted silica for triazolam and its metabolite, α-hydroxytriazolam.

3.4.2. Optimization of the washing step

All the MIP materials in cartridges were washed to disrupt the nonspecific interactions between the analytes and the molecularly imprinted polymer. In this way, the specific interactions with the sorbent would be retained and the analytes could then be quantitatively recovered in the subsequent elution step. 1 mL of methanol/water (1/9, v/v) solution with 2 mg L^{-1} triazolam was passed through the imprinted and non-imprinted silica cartridges. All triazolam was bound by the imprinted silica, while only about 0.6 μg of triazolam was bound by the non-imprinted silica. It has been shown previously that MIPs often exhibit higher selectivity in the solvent used as the porogen in the polymerization [31]. Therefore, 1 mL of sample was passed through the cartridges followed by a washing step, carried out with either water or one of the various ratios of methanol to water, followed by an elution step with methanol. Fig. 7 shows the mass of triazolam in the washing fractions after loading on the imprinted and non-imprinted silica cartridges. According to Fig. 7, triazolam was totally retained on the imprinted silica cartridges when the washing step was carried out with either water or water/methanol (9/1, v/v). About 100% of the triazolam loaded on the non-imprinted silica cartridges was removed by washing with either 4 mL water or 3 mL water/methanol (9/1, v/v). Water/methanol (9/1, v/v) is able to disrupt the nonspecific interactions occurring between the triazolam and the imprinted silica as well as to retain the spe-

Table 2
Separation factors of the target molecules on the imprinted silica column.

	Triazolam	Estazolam	Alprazolam	Clonazepam	Nitrazepam	Diazepam	Chlordiazeposide	Barbital	Pentobarbital
a_{10}	1.1	1.0	1.1	1.0	1.0	1.0	1.0	1.2	1.0
a_{91}	1.3	1.2	1.3	1.0	1.1	1.2	1.2	1.2	1.0
a_{73}	3.1	2.8	3.1	2.1	2.2	3.3	1.9	1.5	1.0

k_i: retention factors of the target molecules on the imprinted silica using $NaNO_2$ as void marker; k_t: retention factor of pentobarbital on the imprinted silica at various mobile phases; a: separation factor calculated as $a = k_i/k_t$; a_{10}: retention factors of the analytes on the imprinted silica using methanol as mobile phase; a_{91}: retention factors of the analytes on the imprinted silica using methanol/water (1/9, v/v) as mobile phase; a_{73}: retention factors of the analytes on the imprinted silica using methanol/water (3/7, v/v) as mobile phase.

Table 3
Binding capacity of the analytes for the imprinted and non-imprinted silica.

Sorbent	The binding capacity of the analytes (μg g^{-1})				
	Triazolam	α-hydroxytriazolam	Alprazolam	Nitrazepam	Phenobarbital
Imprinted	130	124	78	30	34
Non-imprinted	23	16	62	13	29

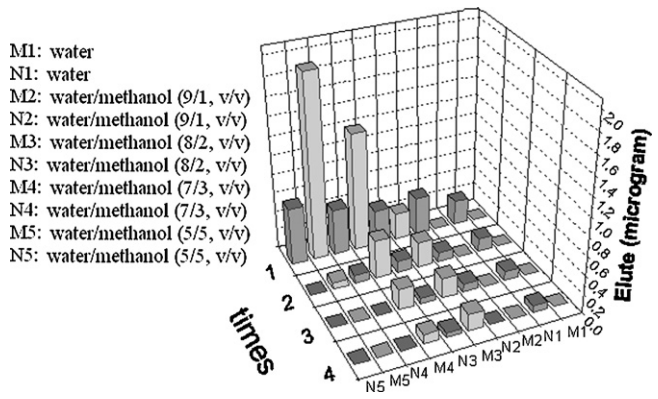

Fig. 7. Mass of triazolam in the washing fractions after loading 1 mL of a 2 mg L^{-1} methanol/water (1/9, v/v) solution of triazolam. Washing step: 1 mL of solvent once for 4 mL solvent.

cific interactions between triazolam and the recognition sites in the imprinted silica. Methanol may be able to remove the water-insoluble interfering agents present in complex samples. For this reason, water/methanol (9/1, v/v) was selected as the washing solvent for all subsequent experiments.

3.4.3. Analytical performance of MISPE in urine samples

The chromatogram of blank urine samples spiked with triazolam and α-hydroxytriazolam is provided in Fig. 8. Compared with the urine samples analyzed directly by HPLC, the same urine samples with MISPE show a very clean chromatographic trace and the peaks corresponding to triazolam and α-hydroxytriazolam can be detected easily.

The calibration curves (i.e., peak area ratios of each examined compound to the internal standard against the amount of each examined compound added) show good linearity in the concentration ranges of 0.2–4.0 μg mL^{-1} urine. The linearity correlation coefficient (r) was 0.983 for triazolam and 0.981 for α-hydroxytriazolam. The percentages recovered at 0.2 and 1.0 μg mL^{-1} were 94 ± 2.3% and 86 ± 4.2%, respectively, for triazolam and 75 ± 2.6% and 80 ± 7.9%, respectively, for α-hydroxytriazolam. The limits of detection (LOD) for triazolam and α-hydroxytriazolam were 30 ± 0.21 ng mL^{-1} and 33 ± 0.26 ng mL^{-1}, respectively. Thus the imprinted silica microspheres exhibited good selectivity for both the template and its main metabolite (α-hydroxytriazolam). This group-selective binding character of the imprinted silica would allow accurate quantification of triazolam and its metabolites.

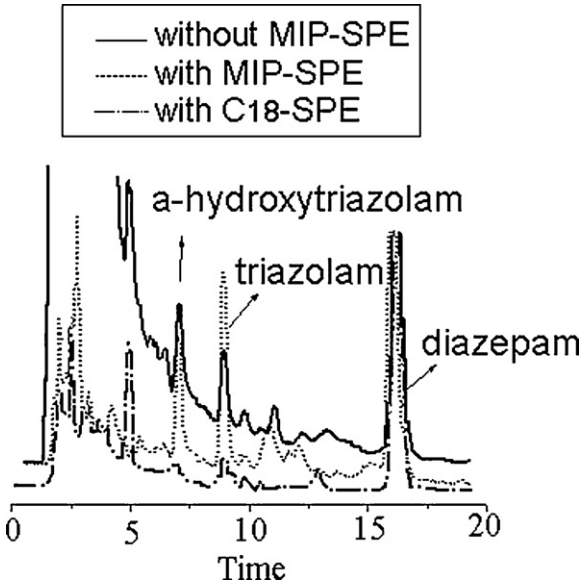

Fig. 8. Sections of HPLC–UV chromatograms obtained before and after extraction of urine with a MISPE cartridge.

4. Conclusion

A silica-supported sol–gel polymer microsphere was synthesized following a surface molecular imprinting protocol using 3-aminopropyltriethoxysilane and phenyltrimethoxysilane as functional monomers and triazolam as the template molecule. The imprinted silica was applied as sorbent in SPE of the urine samples prior to C_{18}-HPLC. The triazolam-imprinted silica exhibited excellent selectivity and affinity for triazolam and its metabolite, α-hydroxytriazolam. The limits of detection for triazolam and α-hydroxytriazolam in urine samples were 30 ± 0.21 ng mL^{-1} and 33 ± 0.26 ng mL^{-1}. Therefore, this MISPE method appears to be well-suited for the removal of the interfering compounds and the enrichment of the target molecules in urine samples prior to chromatographic analysis.

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