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Synthesis and characterization of a molecularly imprinted polymer and its application as SPE enrichment sorbent for determination of trace methimazole in pig samples using HPLC-UV

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ABSTRACT

A novel molecularly imprinted polymer that could be applied as enrichment sorbent was prepared using methimazole (MMZ) as the template molecule, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker. Though evaluated by static, kinetic and competitive adsorption tests, the polymer exhibited high adsorption capacity, fast kinetics and good selective ability. A method for determination of trace MMZ was developed using this polymer as enrichment sorbent coupled with high performance liquid chromatography focusing on complex biological matrices. Under the optimum experimental conditions, the MMZ standard is linear within the concentration range studied, that is, from 0.5 $\mu g\,L^{-1}$ to 150 $\mu g\,L^{-1}$ ($r^2 = 0.9941$). Lower limits of detection (LOD, at S/N = 3) and quantification (LOQ, at S/N = 10) in pig samples were 0.63 $\mu g\,kg^{-1}$ and 2.10 $\mu g\,kg^{-1}$ for kidney, 0.51 $\mu g\,kg^{-1}$ and 1.70 $\mu g\,kg^{-1}$ for liver, 0.56 $\mu g\,kg^{-1}$ and 1.86 $\mu g\,kg^{-1}$ for muscle, respectively. Recoveries and relative standard deviation (RSD, $n = 9$) values for precision in the developed method were from 71.14% to 88.41% and from 2.53% to 6.18%.

1. Introduction

Methimazole (1-methyl-2-mercaptoimidazole, tapazole, MMZ), termed as antihormone, was a model substance for endocrine disruption in physiological and genomic studies and widely used in medicine for treatment of hyperthyroidism [1,2,3]. Its action is to slow iodide integration into tyrosine and thus inhibits the production of thyroid [4,5]. Substantial portion of orally taken drug would be excreted 79.2% in prototype with urine, but the rest would be resorted in tissues [6], which makes it potentially hazardous to human health. It was reported that MMZ could be further metabolized to *N*-methylimidazole and sulfite, via sulfenic (R-SOH) and sulfinic ($R\text{-}SO_2H$) acid intermediates that are associated with the cytotoxic effects of MMZ [6,7]. MMZ side effects may be caused such as irritation of skin, impaired taste, olfaction, allergies or pharyngitis with fever, and in rare occasions, nephritis and liver cirrhosis [7–9]. Thus it is of great importance to determine MMZ for human health.

So far, various analytical methods in TLC [10], GC–MS [11,12], HPLC-UV [4,13,14] or MS [15,16] and CE [6,17] have been described for the effectively monitoring and detecting MMZ in various samples including urine [4,11–14], plasma [10], tissues [4,15,16] and serum [17]. Flow injection analysis [17] and electrochemical detection [8,18,19] for MMZ were also developed. But the sensitivity in these methods needs to be improved. When coupled with MS, sensitive determination could be provided at the low level of MMZ, but it is very expensive and not suitable for extensively prevalence. It is very significant to develop a simple, inexpensive and sensitive analytical method to improve the determination of MMZ in biological samples.

In developed analytical methods, the preparation of biological samples is a very important step. And solid-phase extraction (SPE) was usually utilized to purify various biological systems and preconcentrate the analytes to improve the analytical sensitivity due to the advantages of simplicity, rapidness and less consumption of organic solvents [20–24]. Molecular imprinting technology (MIT) has been proved to be an efficient method to produce functionalized materials which are used as enrichment sorbent of SPE to recognize the template from closely related compounds [22–27].

Abbreviations: MMZ, methimazole; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; SPE, solid-phase extraction; HPLC, high performance liquid chromatography; UV, ultraviolet-visible; MSPD, matrix solid-phase dispersion; IADS, iodine-azide detection system; PMT-SWV, potentiometric titration with square wave voltammetry; MIT, molecularly imprinting technique; MIP, molecularly imprinted polymer; NMIP, non-molecularly imprinted polymer; MAA, methacrylic acid; EGDMA, ethylene glycol dimethacrylate; AIBN, 2,2′-azoisobutyronitrile; DDW, doubly deionized water; ESM, electronic supplementary material.

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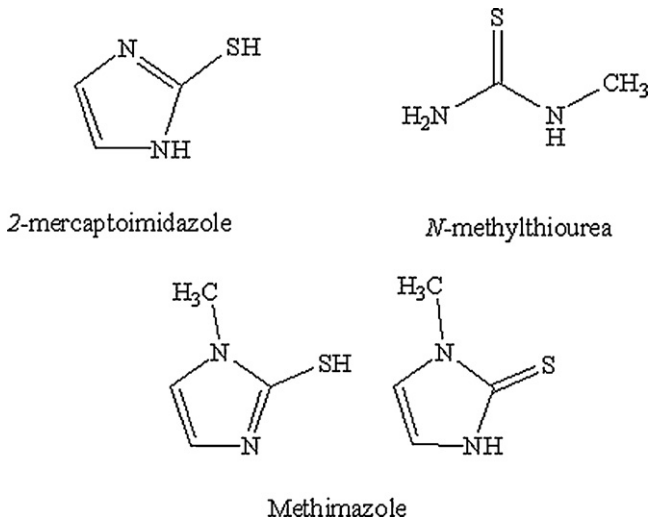

Fig. 1. The structures of MMZ, *N*-methylthiourea and 2-mercaptoimidazole.

In this paper, a novel molecularly imprinted polymer (MIP) is prepared using MMZ as the template, methacrylic acid (MAA) as the monomer, ethylene glycol dimethacrylate (EDMA) as the cross-linker in ethanol solvent with 2,2′-azoisobutyronitrile (AIBN) as free radical initiator. The MMZ-imprinted polymer was evaluated and applied as enrichment material of SPE-HPLC. The parameters of SPE procedure affecting the preconcentration and separation of the analyte was discussed in detail to build a simple, inexpensive, sensitive method as a potential analytical strategy to monitor the reminder MMZ in biological samples.

2. Experimental

2.1. Materials and reagents

MMZ (99.7%, tapazole) was purchased from Beijing Yanjing Pharmaceutical Co., Ltd. (Beijing, China). And pure standard of *N*-methylthiourea and 2-mercaptoimidazole (Fig. 1) were purchased from Sigma–Aldrich. Other chemicals used for the polymer synthesis were the functional monomer MAA (99%, Tianjin Chemical Reagent Research Institute, Tianjin, China) and the cross-linker EDMA (98%, Sigma–Aldrich) and the free radical initiator AIBN (99%, Tianjin Kermel Chemical Reagents Co., Ltd. Tianjin, China).

HPLC-grade solvents used to prepare the LC-mobile phase were methanol purchased from Concord Co., (Tianjin, China) and doubly deionized water (DDW, 18.2 MΩ cm^{-1}) obtained from a Water Pro water system (Labconco Corp., Kansas City, MO, USA). The mobile phase consisted of methanol and 0.02 mol L^{-1} KH_2PO_4 solution (controlled pH 3.2 by H_3PO_4) at the ratio of 3:7 (v/v). Ethanol, acetonitrile and other chemicals used in this experiment were purchased by Tianjin No. 1 Chemical Reagent Factory (Tianjin, China) and of highest available purity and at least of analytical grade.

Individual stock solution of the analytes (MMZ, *N*-methylthiourea and 2-mercaptoimidazole) were initially prepared at 300 mg L^{-1} by dissolving 30 mg of each compound in 100 mL of ethanol and stored at 4 °C in dark. The corresponding working standard solutions were obtained by dilution of the individual stock solutions in further research.

The functional monomer MAA was distilled under reduced pressure. The cross-linker EGDMA from Sigma was extracted with 10% NaOH solution, and dried over anhydrous magnesium sulfate and subsequently distilled under reduced pressure. The AIBN was recrystallized from ethanol and stored at 4 °C.

2.2. Instrumentation

HPLC system consisted of two LC-10ATVP pumps and a Shimadzu SPD-10AVP ultraviolet detector (Shimadzu, Kyoto, Japan). All separations were achieved on an analytical Hypersil-ODS column (4.6 mm × 250 mm long, Thermo) at a mobile flow rate of 0.5 mL min^{-1} at the room temperature. The detection wavelength and injection volume were 254 nm and 20 μL, respectively. Class-VP software was used to acquire and process spectral and chromatographic data. A SPE system (Bellefonte, PA, USA) was applied to the procedure of preconcentration. A Cary 50-Bio UV spectrophotometer (Bio-Rad, USA) and an ultrasonicator were also employed in the adsorption test (λ = 254 nm) and the preparation of HPLC mobile phase, respectively. Adsorption/desorption analyses were carried out using a nitrogen surface area analyzer (Beishide, Beijing, China).

2.3. Preparation of imprinted polymer of MMZ

The pre-polymerization solution, consisting of MMZ (0.25 g, 2.19 mmol), MAA (0.745 mL, 8.78 mmol), EGDMA (2.40 mL, 12.68 mmol), AIBN (0.13 g, 0.79 mmol) and 10 mL ethanol as porogen was prepared in a 50 mL PTFE flask. The mixture was sonicated for 20 min and deoxygenated with a stream of nitrogen gas for 15 min. The flask was sealed and irradiated by UV irradiation (λ = 365 nm) for 12 h. After the polymerization, the resulting polymer was crushed into powder. Fine particles ranging from 80 μm to 125 μm were collected and mixed with 50 mL of methanol and 50 mL of 1.0 mol L^{-1} HCl under magnetically stirring for 2 h to remove the template. The mixture was isolated by filtration, neutralized with 0.1 mol L^{-1} NaOH solution and washed with pure water. Prior to drying, the solid product was extracted by extensive washing with methanol/acetic acid (9/1, v/v), followed at 254 nm with a spectrophotometer until no more template molecule could be detected. And then the polymer was dried in a vacuum at 70 °C for 10 h.

For comparison, a non-molecularly imprinted polymer (NMIP) was prepared in an identical method without adding the template molecule during polymerization.

2.4. Adsorption test

To measure the adsorption capacities, 50 mg of MIP or NMIP was mixed with 10 mL of ethanol solution in different concentrations (10–150 mg L^{-1}), respectively. The mixture was mechanically shaken for 4 h at room temperature and then separated centrifugally (3500 rpm) for 10 min. The supernatant was measured for the unrestricted MMZ by UV spectrometry at 254 nm. The adsorption capacity (Q) was calculated according to the equation being

$$Q = (C_i - C_f)V/W$$

where C_i, C_f is the initial and final concentration of the analytes in the solution, respectively, V, W is the cubage of solution and the mass of polymer, respectively.

Kinetics of the imprinted functionalized polymer was examined at 60 mg L^{-1} MMZ-ethanol solution onto 50 mg of the sorbent. The mixture was mechanically shaken for different time periods (10–120 min) at room temperature and determined by HPLC-UV at 254 nm.

The selectivity of imprinted polymer was validated by selecting *N*-methylthiourea and 2-mercaptoimidazole as the competitors with similar structure and characteristics. The MMZ-imprinted polymer or NMIP (50 mg) was added to a flask containing 10 mL of 60 mg L^{-1} ethanol mixed solution, shaken at room temperature

for 4 h and separated centrifugally. The supernatant was diluted and determined by HPLC-UV.

2.5. Procedure of SPE

In order to face the sensitive and quantitative determination of MMZ at the required levels, a SPE procedure was carried out using this imprinted polymer as enrichment sorbent.

SPE procedure was performed using a Visiprep™-DL SPE vacuum manifold from Supelco (Bellefonte, PA, USA). Empty SPE tubes were packed with 50 mg of imprinted or non-imprinted polymer using two PTFE frits at each end. SPE cartridges were preconditioned with 5 mL of DDW and 5 mL of methanol to activate the sorbent before the enrichment procedure. 100 mL of MMZ-ethanol solution were uploaded onto the preconditioned cartridge. After loading, the vacuum was still applied to the cartridges for 30 min in order to remove the residual solvent. 1.5 mL of ethanol/water (8/2, v/v) were added to the cartridges to eliminate molecules retained by physical adsorption by the polymer. Eluting step was performed using 5.0 mL of methanol/acetic acid (9/1, v/v) mixture solution. The elution fractions were dried under a gentle nitrogen stream. The residue was dissolved with 1 mL of methanol and filtered through a 0.45 μm nylon filter for subsequent HPLC analysis.

2.6. Preparation of samples

Fresh pig samples (kidney, liver and muscle), were chosen as biological matrices to evaluate the usefulness of the developed method. 5 g of tested samples, determined to be free of the analyte, was crushed up and added into a 50 mL polypropylene centrifuge tube, spiked with three concentration levels containing 0.05 μg, 0.1 μg, 0.15 μg of MMZ, respectively. After 10 min rest in the dark, 10 mL acetonitrile which was used to extract the analyte and precipitate the protein was added. The mixture was consumingly shaken for 3 min by the vortex machine, separated by a centrifuge at 3500 rpm for 5 min at 4 °C.

The residues were extracted repeatedly with the same procedure. The two part supernatants of acetonitrile were incorporated and mixed 30 mL saturated hexane in order to remove the fat of the samples. After shaking for 3 min, the acetonitrile was separated, dried with unhydrous sodium sulfate and the residue was washed with 2 mL of acetonitrile.

The product was evaporated at 40 °C with a rotary evaporation. The dried acetonitrile extract was dissolved with ethanol, transferred into a 100 mL calibrated flask and diluted to mark for analyzing with the developed method of SPE coupled with HPLC.

3. Results and discussion

3.1. Preparation conditions of MMZ-MIP

The preparation process of MMZ-MIP is quite simple, but a number of factors which affect the polymerization and imprinted effect have to be taken to account. For the porogen, it is required to be soluble of the template, monomer, cross-linker and initiator. And its relatively low polarity is advantageous to reduce the interferences between imprint molecule and the monomer during complex formation, as the latter is very important to obtain high selectivity MIPs. In this experiment, methanol, acetonitrile, and ethanol were tested to select appropriate porogen. Although the reactants can be dissolved in these solvents, the MIP synthesized in ethanol affords better binding affinity than in other solvent. Also considered its lower toxicity, ethanol was chosen as the porogen during the preparation of MIP. Monomers with different character (MAA and acrylamide), which may form hydrogen bonding with MMZ, were used in the choice of the functional monomer. By comparison, MAA showed to be more suitable to bind MMZ and was selected as the functional monomer in the MMZ-MIP preparation.

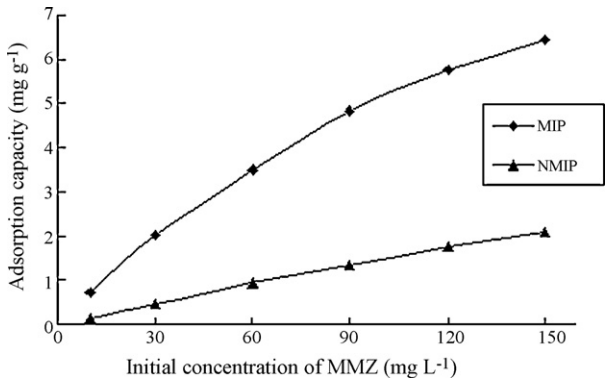

Fig. 2. Binding isotherms of MIP and NMIP for MMZ in ethanol.

Another important parameter that affected the capability of MIP synthesized in the synthesis process is molar ratio of template/monomer/cross-linker. Only appropriate molar ratio of template/monomer can afford high selectivity with MIP. And the amount of cross-linker should be high enough to maintain the stability of the recognition sites [28,29]. Through complex optimization experiments, the MIP synthesized at the molar ratio of 1:4:5.81 (template/monomer/cross-linker) in 10 mL of ethanol showed better affinity and selectivity and were chosen as synthetical conditions of MMZ-MIP.

3.2. Evaluation of static adsorption

The isothermal adsorption results (Fig. 2) showed that the adsorption capacity of MIP or NMIP toward template molecule increased with the increasing initial concentration. And under the same experimental conditions, the adsorption capacity of the imprinted polymer at each concentration was higher than three times that of the non-imprinted polymer. This indicated that MIP offered a higher affinity for the template molecule than NMIP.

The Scatchard model was also used for evaluation of the adsorption of imprinted polymer, the equation [30,31] being

$$\frac{Q}{C_i} = \frac{-Q}{k} + \frac{Q_{max}}{k}$$

where C_i is the initial concentration of the analytes in the solution, k is equilibrium dissociation constant, Q is the adsorption capacity at adsorption equilibrium, and Q_{max} is the saturated adsorption capacity.

From the equation, Q/C_i versus Q is plotted in Electronic Supplementary Material (ESM) (A). The results indicated that the adsorption isotherm of MMZ-imprinted polymer towards the template molecules was in good accordance, with linearity ($r^2 = 0.9933$) under the experimental conditions. The saturated adsorption capacity (Q_{max}) of imprinted sorbent toward MMZ was 14.9 mg g^{-1} obtained by the linear slope (k^{-1}). Results from Scatchard analysis showed that the imprinted polymer had higher binding association constants and more apparent binding sites than non-imprinted sorbent. Some parameters affecting the efficiency of adsorption including the specific surface area and pore volume were also evaluated in nitrogen adsorption experiment. The specific surface area, pore volume and pore size with MIP were 92.46 m^2 g^{-1}, 0.1911 m^3 g^{-1}, 8.319 nm, respectively, which were similar to those with NMIP (91.90 m^2 g^{-1}, 0.1858 m^3 g^{-1}, 8.038 nm). This demonstrated that the binding affinity of the imprinted polymer was mainly from the specific sites formed by the imprinting effect. And all these characteristics indicated that the synthesized MIP was

Table 1
Competitive loading of MMZ, 2-mercaptoimidazole and *N*-methylthiourea by MIP and NMIP.

Sorbents		MIP	NMIP
Loading capacity ($mg\,g^{-1}$)	MMZ	3.17	0.89
	2-Mercaptoimidazole	0.83	0.67
	N-methylthiourea	1.18	0.92
[a]K_d	MMZ	179.15	40.04
	2-Mercaptoimidazole	37.13	29.35
	N-methylthiourea	54.60	41.63
[b]K	2-Mercaptoimidazole	4.82	1.36
	N-methylthiourea	3.28	0.96
[c]K'	2-mercaptoimidazole	3.54	
	N-methylthiourea	3.41	

[a] K_d, distribution coefficient; $K_d = \{(C_i - C_f)/C_f\} \times \{\text{volume of solution (mL)}\}/\{\text{mass of gel (g)}\}$. Where C_i and C_f represent the initial and final concentrations, respectively.
[b] K, selectivity coefficient, $K = K_d$ (MMZ)/K_d (2-mercatoimidazole or *N*-methylthiourea)
[c] K', relative selectivity coefficient, $K' = K_{MIP}/K_{NMIP}$

potential to be used for selective enrichment and detection of MMZ from sample matrix.

3.3. Uptake kinetics of MMZ by the imprinted polymer

The kinetic adsorption test results of the MMZ-imprinted sorbent ($60\,mg\,L^{-1}$ MMZ onto 50 mg of the imprinted polymer) were shown in Electronic Supplementary Material (ESM) (B). More than 50% of binding was obtained within a short shaking period of 30 min, and the adsorption equilibrium was almost reached within 60 min. By decreasing the concentration of MMZ, the time to saturation would become shorter. These results indicated that the MIP held faster uptake kinetics and can be used as enrichment sorbent of SPE for rapid determination.

3.4. Selectivity of the imprinted polymer for MMZ

The results of selectivity test of the MIP and NMIP towards MMZ, 2-mercaptoimidazole and *N*-methylthiourea in $60\,mg\,L^{-1}$ ethanol mixed solution were shown in Electronic Supplementary Material (ESM) (C). Compared to the loading capacity of NMIP ($0.89\,mg\,g^{-1}$), a higher capacity was achieved to $3.17\,mg\,g^{-1}$ of MIP towards MMZ. The loading capacities of 2-mercaptoimidazole ($0.83\,mg\,g^{-1}$) and *N*-methylthiourea ($1.18\,mg\,g^{-1}$) under the same condition were much lower than that of MMZ in MIP. While with the NMIP, they (0.67 and $0.92\,mg\,g^{-1}$) showed no obvious differences from the MMZ ($0.89\,mg\,g^{-1}$). The parameters including distribution coefficient (K_d), selectivity coefficient (K) and relative selectivity coefficient (K') of MIP and NMIP towards three analytes were calculated according to the loading capacities of the analytes (Table 1). The large K value of MIP (4.82 and 3.28) indicated its high selectivity for MMZ over other compounds. The imprinting effect for MMZ was revealed as the increasing K and K' (3.54 and 3.41) through the comparison K value of MIP with the corresponding NMIP (1.36 and 0.96). The results of K' also indicated that the imprinted effects for 2-mercaptoimidazole and *N*-methylthiourea of MIP because of their related structures from MMZ. These results might root in that during the preparation of the MIP, MMZ, as the template of synthetic reactivity, was incorporated in inorganic network. The specific imprinted cavities and binding sites were formed in MIP after the removal of MMZ and imprinted effect emerged when other competitors existed in the solution.

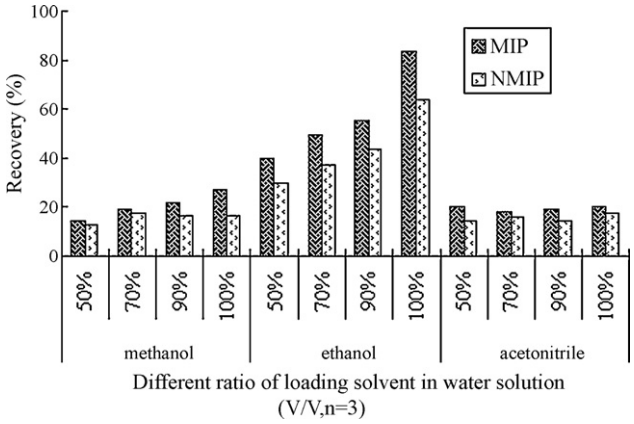

Fig. 3. Recoveries of MMZ in SPE procedure with different sample loading solvent.

3.5. Optimization of SPE procedure

In order to evaluate the applicability of the MIP for separation and determination of MMZ by HPLC, general parameters for SPE including the type and amount of loading solvents, washing solution and eluent were optimized following a step by step approach.

Sample loading solvent plays an important role in the enrichment of the analyte due to the fact that it determines the microenvironment of the binding reaction and simultaneously influences the stability of analyte [24]. In order to evaluate the effect of loading solvent in SPE procedure, different loading solvents such as methanol, ethanol, acetonitrile, methanol/H_2O (50%, 70%, 90%, v/v), ethanol/H_2O (50%, 70%, 90%, v/v), acetonitrile/H_2O (50%, 70%, 90%, v/v) were investigated on the MIP and NIP column. The optimum results (Fig. 3) were obtained when ethanol was employed, which almost all the loaded analyte was retained by the MIP cartridge with recovery being 83.87%. This might be that ethanol as the porogen solvent in polymerization, which could influence the degree of polymer chain solvation and adjusted the solvation of microenvironment of the binding sites similar within the developing polymer [32]. Compared to ethanol, the binding results in methanol, acetonitrile and water-containing solvents were obviously affected by the polarity of solvents, which had a direct disruption for the specific interaction between the template molecule and the MIPs, which led to a significant loss in loading step for MIP cartridge. As is shown in Fig. 3, the recovery of each NMIP column was lower than that on MIP column under the same experimental conditions, which also proved that specific interaction between the specific sites and the analyte exactly existed during the binding procedure. Different volume (50 mL, 100 mL, 150 mL, 200 mL and 250 mL) of $1.0\,\mu g\,L^{-1}$ MMZ-ethanol solution was loaded onto the cartridges that contained 50 mg MIP of MMZ. According to the compared data obtained under the same condition, 100 mL $1.0\,\mu g\,L^{-1}$ MMZ-ethanol solution was selected as the sample loading condition in subsequent experiments.

Washing step was a crucial procedure to maximize the specific interactions between the analytes and binding sites, and to simultaneously decrease non-specific interactions to discard matrix components of the polymer [33]. In this part, ethanol–H_2O solution of different ratio (19:1, 9:1, 8:2, 7:3 and 5:5, v/v) and different volume (1 mL, 2 mL, 3 mL) was investigated on the MIP and NMIP cartridges (Fig. 4). When 1 mL washing mixture of ethanol and H_2O was at the ratio 19:1 and 9:1, no obvious effect was caused on the retention of MMZ on the sorbent with the recoveries being (MIP: 88.50% and 86.82%, NMIP: 69.89% and 66.16%). With the increase of H_2O in the 1.0 mL washing solution (8:2), the recovery from the NMIP sorbent was decreased rapidly to (54.56%), while the recovery from the MIP sorbent was not reduced (88.18%). In other words,

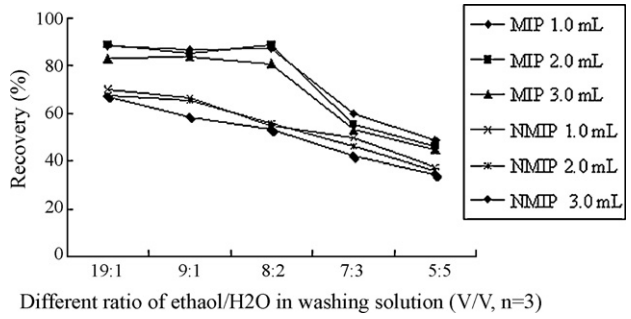

Fig. 4. Recoveries of MMZ on MIP and NMIP column with different washing solution.

the ethanol-H_2O solution (8:2) could decrease non-specific interactions, but do not affect the interaction between the analyte and binding sites. However, the higher portion of H_2O (7:3 and 5:5) led to the large decrease of the analyte on both MIP (59.85% and 48.69%) and NMIP sorbent (49.89% and 37.57%). That might result from the disruption of specific interactions between the analyte and binding sites caused by the changed polarity of washing solution. The recoveries on MIP and NMIP column were in small difference when using ethanol–H_2O at the ratio of 7:3 and 5:5 as the washing solution. It was because that the MIP and NMIP sorbent held similar specific surface areas, which could bring about similar non-specific interaction to the analyte. By comparison of the obtained data of different washing volumes, the results showed that the recovery of MIP with 1 mL ethanol–H_2O solution (8:2) (86.82%) was almost equal to that with 2 mL washing solution (85.25%), but higher than that with 3 mL (80.67%) as the washing condition. Based on the results above, 1.5 mL ethanol–H_2O solution (8:2) was chosen as the washing condition in further research.

An appropriate eluent should be chosen to ensure the analyte can be completely eluted from the MIP cartridge. For this purpose, different type of solvents including H_2O, methanol, H_2O/methanol (9:1 and 7:3, v/v), methanol/acetic acid (9:1, 7:3 and 5:5, v/v), ethanol/acetic acid (9:1, 7:3 and 5:5, v/v), H_2O/$NH_3{\cdot}H_2O$ (9:1, v/v), methanol/$NH_3{\cdot}H_2O$ (9:1, v/v), were applied and compared for the selection of the final eluent (Fig. 5). Among the tested solvents, acetic acid-containing eluent offered the higher recovery than other types of solvents, and in the case of methanol/acetic acid (9:1) as the eluent, the highest recovery was achieved (more than 90%). The solvents containing methanol and H_2O could almost achieve the same recovery (65–75%). These results proved that the acidity of the eluent is of more importance than the polarity in the desorption procedure between the template molecule and the MIP.

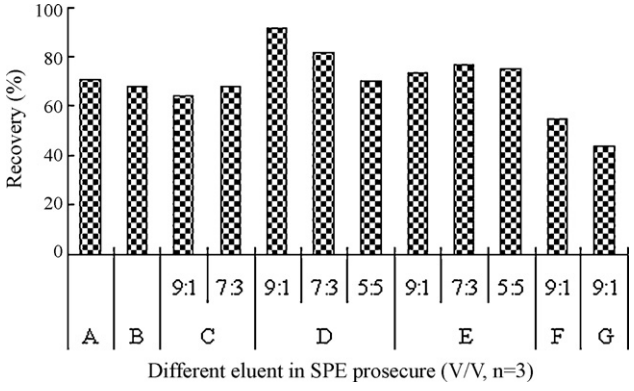

Fig. 5. Recoveries of MMZ in SPE with different eluent (A) H_2O (B) methanol (C) H_2O/methanol (D) methanol/acetic acid (E) methanol/acetic acid (F) H_2O/$NH_3{\cdot}H_2O$ (G) methanol/$NH_3{\cdot}H_2O$.

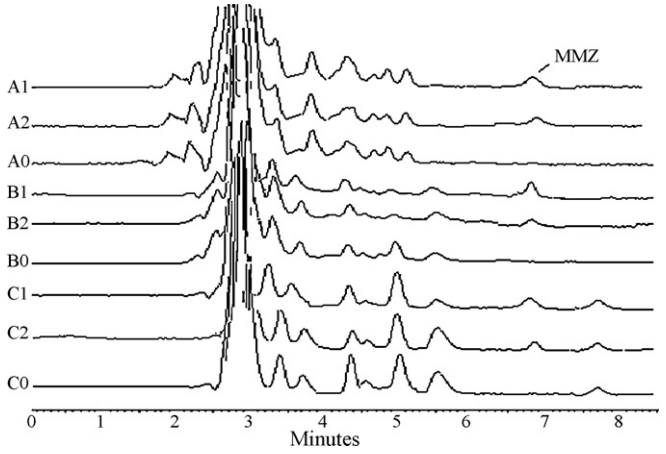

Fig. 6. Chromatograms of MMZ using MIP and NMIP as SPE enrichment sorbent for 5 g of pig kindey (MIP: A1, NMIP: A2, non-spiked: A0), liver (MIP: B1, NMIP: B2, non-spiked: B0) and muscle (MIP: C1, NMIP: C2, non-spiked: C0) samples spiked at 10 $\mu g\,kg^{-1}$ concentration.

The eluent volume is also a crucial parameter to be optimized in SPE. The chosen volume of eluent must be just sufficient to elute the analyte from the sorbent. Thus, recoveries of MMZ were studied in applying different eluent volumes of 1.0–8.0 mL. The results indicated that good recoveries were achieved at 5.0 mL of methanol/acetic acid (9:1), and more volume provided similar recovery values, which showed that 5 mL of methanol/acetic acid (9:1) was enough to provide a quantitative elution of the analyte from the sorbent.

3.6. Molecularly imprinted solid-phase extraction and determination of MMZ in pig samples

Linearity was achieved at the concentration range between 0.5 $\mu g\,L^{-1}$ and 150 $\mu g\,L^{-1}$ in MMZ-ethanol solution through the SPE procedure with good correlation coefficients ($r^2 = 0.9941$). Under the optimum conditions of SPE, limits of detection (LOD, S/N = 3) and quantification (LOQ, S/N = 10) in samples were achieved to 0.63 $\mu g\,kg^{-1}$ and 2.10 $\mu g\,kg^{-1}$ for kidney, 0.51 $\mu g\,kg^{-1}$ and 1.70 $\mu g\,kg^{-1}$ for liver, 0.56 $\mu g\,kg^{-1}$ and 1.86 $\mu g\,kg^{-1}$ for muscle, respectively. Chromatograms of MMZ for 5 g of pig samples spiked at 10 $\mu g\,kg^{-1}$ concentration through MIP or NMIP SPE procedure were shown in Fig. 6. Compared to the recoveries being between 41.64% and 64.62% on NMIP column, higher recoveries at three spiked levels (10 $\mu g\,kg^{-1}$, 20 $\mu g\,kg^{-1}$, 30 $\mu g\,kg^{-1}$) in free pig samples were obtained ranging from 71.14% to 88.41% on MIP column. And the relative standard deviation (RSD, $n = 9$) on MIP and NMIP column was from 2.53% to 6.18% and 1.63% to 6.33%, respectively (Table 2).

Table 2
Recoveries of kidney, liver and muscle samples spiked MMZ (mean (RSD %), $n = 9$).

Pig samples	Spiked level ($\mu g\,kg^{-1}$)	Recovery of MMZ (%)	
		MIP	NMIP
Kidney	10	76.61 ± 4.90	43.26 ± 4.65
	20	84.96 ± 2.65	56.12 ± 1.63
	30	85.51 ± 2.53	64.62 ± 3.86
Liver	10	71.14 ± 6.18	41.64 ± 3.89
	20	83.62 ± 4.28	55.74 ± 6.33
	30	87.05 ± 4.09	58.05 ± 1.76
Muscle	10	76.90 ± 3.50	42.50 ± 4.02
	20	86.06 ± 4.39	54.11 ± 3.76
	30	88.41 ± 2.81	63.84 ± 4.36

Table 3
Comparison of analytical parameters for the determination of MMZ.

Methods	LOD	Recoveries	RSD	Application samples	Number
MSPD-GC/MS	4 μg g^{-1}	Above 70%	2.1–7.9%	Milk and urine	[11]
	0.05 μg g^{-1}	47.3–63.8%	4.5–8.7%	Pig muscle, thyroid, liver and beef	[12]
MMSPE-HPLC	60 μg L^{-1}	79.9–97.4%	4.8–6.9%	Fish	[4]
RP-HPLC	150 μg L^{-1}	95.7–103.3%	1.8–5.0%	Urine	[13]
IADS-HPLC	114.2 μg L^{-1}	97–102%	0.3–3.2%	Urine	[14]
HPLC–MS	0.023 μg L^{-1}	93–102%	1.2–7.7%	Poultry liver, muscle and egg	[16]
PMT-SWV	57 μg L^{-1}	96.80%	2.9%	Drug tablet	[19]
SPE-HPLC	0.63 μg kg^{-1}, 0.51 μg kg^{-1}, 0.56 μg kg^{-1}	71.14–88.41%	2.53–6.18%	Pig kidney, liver and muscle	This article

4. Conclusions

In this paper, a MIP of MMZ was synthesized by bulk UV polymerization for the separation and preconcentration of MMZ in ethanol solvent. Through evaluated in a series of adsorption experiments, the polymer exhibited good recognition and selective ability, suggesting that it could be a useful tool for analytical purposes. Furthermore, a method was successfully developed to detect MMZ at low concentration levels in biological samples using this MIP as enrichment sorbent of SPE coupled with HPLC. Compared to the methods used for determining MMZ in different samples (Table 3), the method developed in this article exhibited better characteristics such as sensitivity and facility. It should focus on the MMZ determination in other samples in further research. This paper also offered a new method to determine other analytes in different samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.04.004.

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