Preparation and Evaluation of Molecularly Imprinted Polymers for Promazine and Chlorpromazine by Multi-step Swelling and Polymerization: the Application to Determination of Promazine in Rat Serum by Column-switching LC

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Abstract

Molecularly imprinted polymers (MIPs) for promazine (PZ) and chlorpromazine (CPZ) were prepared by multi-step swelling and polymerization using methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a crosslinker. Their retention and molecular-recognition properties for PZ and CPZ were evaluated using a mixture of potassium phosphate buffer and acetonitrile, or a mixture of ammonium formate and acetonitrile as the mobile phase in LC. PZ and CPZ gave the maximal retentions on MIP\textsubscript{PZ} and MIP\textsubscript{CPZ} at apparent pH 8.2 of a mobile phase using a mixture of potassium phosphate buffer and acetonitrile as the mobile phase. The retentions of PZ and CPZ were decreased with an increase of acetonitrile contents from 70 to 90 vol\% using a mixture of ammonium formate and acetonitrile as the mobile phase. The template molecules (PZ and CPZ, respectively) were recognized the most on the respective MIPs, and the imprinting factors of PZ were higher on MIP\textsubscript{CPZ} than MIP\textsubscript{PZ}. These results indicate that in addition to shape recognition, ionic and hydrophobic interactions seem to work for the retention and molecular-recognition of PZ and CPZ on the MIPs. MIP\textsubscript{CPZ} was successfully utilized for selective extraction of PZ in rat serum samples in column-switching LC with fluorescence detection.

Keywords: Chlorpromazine, Column-switching LC, Molecularly imprinted polymer, Multi-step swelling and polymerization, Promazine.
Introduction

Promazine (PZ), which belongs to phenothiazine antipsychotics, is prescribed for schizophrenia.\(^1\) Phenothiazines having an alkylamino group as a sidechain include PZ, chlorpromazine (CPZ), promethazine (PMZ), a cepromazine (APZ) and levopromazine. Phenothiazines treatment often leads to extrapyramidal symptoms, which are serious side-effects of antipsychotic drugs,\(^2\) and to the high interindividual variability in pharmacokinetic properties.\(^3\) Thus, the monitoring of CPZ concentrations in serum can be performed to maintain them within a therapeutic window.\(^3,4\)

The methods so far employed for the determination of PZ and its metabolites in biological samples include LC with UV\(^5\) or coulometric detection,\(^6\) LC-MS/(MS)\(^7-10\), GC with flame thermionic detection\(^11\) and GC-MS.\(^12\) The sample treatments utilized were protein precipitation,\(^8,9\) solvent extraction\(^5,7,10,11\) and solid-phase extraction (SPE).\(^6,12\) Molecularly imprinted polymers (MIPs), which are synthetic polymers having selective recognition abilities for a template molecule and its analog(s), could be utilized for separation, extraction and sensing as molecular recognition elements.\(^13\) MIP-based SPE could be often used for extraction of a target compound from complex matrices; biological samples such as serum and urine, food samples and environmental samples.\(^14-17\) There is no report for the synthesis of a MIP for PZ, MIP\(_{PZ}\). However, a MIP for CPZ, MIP\(_{CPZ}\), has been prepared by bulk polymerization\(^18,19\) and precipitation polymerization\(^20,21\) methods. Song et al.\(^18\) prepared the MIP using methacrylic acid (MAA) and trimethylolpropane trimethacrylate as a functional monomer and crosslinker, respectively, by bulk polymerization, and applied to the determination of CPZ in pig urine. Figueiredo et al.\(^19\) prepared the MIPs using MAA and ethylene glycol dimethacrylate (EDMA) as a functional monomer and crosslinker, respectively, by bulk polymerization, followed by prepartation of the MIP films. The MIP films allowed the quantitation of five phenothiazines
including PZ and CPZ in human urine. On the other hands, a restricted access MIP capped with bovine serum albumin (BSA) was prepared and applied to the determination of CPZ in human plasma. First, the MIP was synthesized using MAA and EDMA as a functional monomer and crosslinker, respectively, by precipitation polymerization, followed by addition of hydroxy methyl methacrylate and glycerol dimethacrylate. Next, the MIP was capped with BSA using glutaraldehyde as a crosslinker followed by reaction with sodium borohydride. MIPs for phenothiazine (PTZ) and 2-chlorophenothiazine were prepared using MAA, EDMA and chloroform as the functional monomer, crosslinker and porogen, respectively, by precipitation polymerization. Furthermore, PTZ-imprinted polymers were applied to extract PZ, CPZ, APZ or perphenazine in meat (chicken and pork) samples.

Since the conventional pretreatment procedures such as protein precipitation, solvent extraction and SPE are lacking in selectivity, MIP-based extraction procedures have been widely used. One of their disadvantages is a leakage problem, where the leaked template molecule prevents the accurate and precise assay for a target compound. In order to overcome this problem, a structurally related analogue or a deuterated molecule (a dummy-template molecule) has been used to prepare MIPs. In the previous study, we prepared MIPs for warfarin (WF) and coumachlor (4′-chlorowarfarin, CWF), MIPWF and MIPCWF, respectively, using 4-vinylpyridine and EDMA as the functional monomer and crosslinker, respectively, by multi-step swelling and polymerization. WF were retained and recognized more strongly on MIPCWF than MIPWF; that is, the retention and imprinting factors of WF were larger on MIPCWF than MIPWF. Furthermore, we tried to apply MIPCWF as a pretreatment column for the determination of WF in human serum samples in column-switching LC.

In this study, MIPPZ and MIPCpz were prepared using MAA and EDMA as the functional monomer and crosslinker, respectively, by multi-step swelling and polymerization. The retention and molecular-recognition properties of PZ, CPZ, PMZ and PTZ on MIPPZ and MIPCpz
were evaluated using a mixture of potassium phosphate buffer and acetonitrile, or ammonium formate and acetonitrile. Furthermore, MIP_{CPZ} was applied for the determination of PZ in rat serum samples as a pretreatment column in column-switching LC with fluorometric detection.

**Experimental**

**Reagents and chemicals**

CPZ and PMZ hydrochlorides, PTZ, MAA and EDMA were purchased from Tokyo Chemical Industry (Tokyo, Japan). PZ hydrochloride was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Polyvinyl alcohol (degree of polymerization = 500, saponification value = 86.5-89 mol%) and an analytical column (Cosmosil 5C_{18}-AR-II, 150 mm × 4.6 mm I.D.) were purchased from Nacalai Tesque (Kyoto, Japan). 2,2’-Azobis(2,4-dimethyl valeronitrile) (ADVN) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Rat serum was purchased from Cedarlane Laboratories (Burlington, VT, USA). Other reagents and solvents were of analytical-reagent grade and were used without further purification. Water purified with a PURELAB Ultra (Organo, Tokyo, Japan) system was used to prepare mobile phases and sample solutions. The structures of PZ, CPZ, PMZ and PTZ used in this study are shown in Fig. 1.

*Preparation of MIPs by Multi-step swelling and polymerization*

One mol L⁻¹ NaOH solution was added for making CPZ or PZ hydrochloride solution alkaline. Free CPZ or PZ in the alkalized solution was extracted three times with chloroform. After evaporation of chloroform, the obtained solid powder was dried at 50 °C for 5 h in vacuo. MIP_{PZ} and MIP_{CPZ} was prepared using PZ and CPZ, respectively, as a template molecule by
multi-step swelling and polymerization according to the method reported previously. \(^{27}\) Non-imprinted polymer (NIP) was prepared similarly but without template molecule. The charged molar amounts of a template molecule (PZ or CPZ), a functional monomer (MAA) and a crosslinker (EDMA) were 4, 6 and 25 mmol, respectively. The resulting polymer particles were washed, collected and dried as reported previously. \(^{27}\)

*Scanning electron micrograph images and particle diameter measurement*

Scanning electron micrograph (SEM) images were obtained with a Mighty-8 instrument (Technex, Machida, Japan). The average particle diameters of MIPs and NIP were measured from SEM images by ImageJ software (National institute of health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/) in triplicate.

*Porosity measurements*

Prior to measurements of the surface areas of MIPs and NIP, a 200 mg weight of the polymers was heated at 80 °C for 4 h in vacuo. They were measured by nitrogen adsorption porosimetry using a TriStar 3000 surface area and porosimetry analyzer (Micromeritics Instruments, Norcross, GA, USA). The specific surface areas were calculated using the Brunauer–Emmett–Teller (BET) method.

*Evaluation of retention and molecular-recognition properties of MIPs*

For the evaluation of their retention and molecular-recognition properties, MIP\(_{PZ}\), MIP\(_{CPZ}\) or NIP was packed into a stainless-steel column (10 mm × 4.6 mm i.d.) using methanol / 2-propanol (2/1, v/v) and methanol as the slurry and packing solvents, respectively, at constant pressure of 9.8 MPa. The LC system used was an LC-20AD pump, an SPD-20A spectrophotometer, a C-R8A integrator (all from Shimadzu, Kyoto, Japan) and a Rheodyne 7725
injector (Rheodyne, Cotati, CA, USA) with a 20 µ L⁻¹ loop. Column temperature was kept at 25 °C using a Thermo Minder SM-05R water-bath (Taitec, Saitama, Japan), and detection was performed at 255 nm. The flow rate was maintained at 0.3 mL/min.

The effects of mobile phase pH, pH 6.0 − 9.8, on the retention and molecular-recognition properties of PZ, CPZ, PMZ and PTZ on MIPs and NIP were evaluated using 50 mmol L⁻¹ potassium phosphate buffer / acetonitrile (30/70, v/v). In addition, the effect of acetonitrile contents, 70 − 95 vol%, on those of PZ, CPZ, PMZ and PTZ was evaluated using water / acetonitrile including 5 mmol L⁻¹ ammonium formate as the mobile phase. The retention factor \( k \) was calculated using the equation \( k = (t_R - t_0)/t_0 \), where \( t_R \) and \( t_0 \) are the retention times of the retained and unretained solutes, respectively. The retention time of the unretained solute, \( t_0 \), was measured by injecting acetone. The imprinting factor (IF) was calculated using the equation \( IF = k_{MIP} / k_{NIP} \), where \( k_{MIP} \) and \( k_{NIP} \) are the solute k’s on MIP and NIP, respectively.

**Column-switching LC conditions for selective extraction of PZ in rat serum samples**

Operation of LC-20AD and LC-20AB pumps, a RF-20Axs fluorescence detector, a SIL-20AHT autosampler, a CTO-20A column oven and an FCV-12AH switching valve was performed by a CBM-20A system controller using LabSolutions software (all from Shimadzu, Kyoto, Japan). A pretreatment column packed with MIP_{CPZ} (10 mm× 4.0 mm i.d.) was equilibrated with 50 mmol L⁻¹ ammonium formate / acetonitrile (20/80, v/v) at a flow rate of 0.8 mL/min. Rat serum samples spiked with PZ concentrations of 0.02, 0.05, 0.10, 0.50, 1.00, 5.00, 10.0 and 20.0 µg mL⁻¹ and a CPZ concentration of 20 µg mL⁻¹ were deproteinized with the same volume of acetonitrile and centrifuged at 10,000 rpm for 10 min. A 100 µL portion of a deproteinized rat serum sample was loaded onto a pretreatment column. After 3 min, PZ retained on the pretreatment column was transferred to an analytical column [Cosmosil 5C₁₈-AR-II (150 mm × 4.6 mm i.d.) ] in the back-flush mode using water / acetonitrile (70/30, v/v) including 0.5 vol%
formic acid at a flow rate of 0.8 mL min\(^{-1}\). Detection was performed at excitation and emission wavelengths of 315 and 448 nm, respectively. The pretreatment and analytical columns were kept at 30 °C in the column oven. The W-REG 2 software, http://www2u.biglobe.ne.jp/~SA TORU/, was used to create a calibration graph. The intra- and inter-day accuracy and precision data were obtained with the assay of spiked rat serum samples.

**Results and Discussion**

**Preparation of MIP_{PZ} and MIP_{CPZ}**

We prepared monodispersed MIPs for basic compounds, (S)-propranolol\(^{28}\), \(d\)-chlorpheniramine\(^{27,29}\) and \(-brompheniramine\(^{27,29}\) nicotine\(^{30}\) and matrine and oxymatrine\(^{31}\) using MAA as the functional monomer by multi-step swelling and polymerization or precipitation polymerization. Since PZ and CPZ, whose \(pK_a\) values are reported to be 9.48 and 9.41\(^{32}\), respectively, were basic compounds, MIP_{PZ} and MIP_{CPZ} were prepared using MAA and EDMA as the functional monomer and crosslinker, respectively, by multi-step swelling and polymerization according to the method reported previously\(^{27}\).

The particle diameters of MIP_{PZ}, MIP_{CPZ} and NIP are 5.93 ± 0.63, 5.99 ± 0.77, 5.89 ± 0.70 μm, respectively, as shown in Fig. 2. The specific surface areas of MIP_{PZ}, MIP_{CPZ} and NIP are 224, 264 and 246 m\(^2\) g\(^{-1}\), respectively. Both MIPs and NIP gave high size uniformity and large specific surface areas, which suggest the usefulness of the MIPs as LC packing materials and solid-phase extraction media.

**Retention properties of PZ, CPZ, PMZ and PTZ on MIP_{PZ} and MIP_{CPZ}**

Fig. 3A and B shows the effects of mobile phase pH on the retentions of PZ, CPZ, PMZ and PTZ on MIP_{PZ} and MIP_{CPZ}, respectively, where 50 mmol L\(^{-1}\) potassium phosphate buffer /
acetonitrile (30/70, v/v) was used as the mobile phase. PZ, CPZ and PMZ gave the maximal retentions at apparent pH 8.2 of a mobile phase, while the retention of PTZ remained unchanged throughout mobile pHs tested. The retention tendencies of PZ, CPZ, PMZ and PTZ on NIP was similar with those on the MIPs except that the values were especially small for PZ and CPZ, moderately small for PMZ and almost the same for PTZ. These results mean that PZ and CPZ are retained more strongly on the MIPs than the NIP because of molecularly imprinting effects, and that PMZ are recognized moderately on the MIPs but PTZ are not recognized. The pKa values of PZ, CPZ and PMZ were reported to be 9.48, 9.41 and 8.98, respectively. It was reported that the pKa value of L-phenylalanine anilide-imprinted MAA-co-EDMA polymers shifted to 8-9. Furthermore, the maximal retention of L-phenylalanine anilide could be observed at its apparent pKₐ value on the imprinted MAA-co-EDMA polymers based on an ion-exchange process. In our previous study, the maximal retention of d-chlorpheniramine, whose pKₐ value is 9.2, was observed at apparent pH 8.0 in 50 mmol L⁻¹ potassium phosphate buffer / acetonitrile (30/70, v/v) on d-chlorpheniramine-imprinted MAA-co-EDMA polymers. This was due to the shift of apparent pKₐ value of d-chlorpheniramine in a mixture of potassium phosphate buffer and acetonitrile. Thus, the retention data of PZ, CPZ, PMZ and PTZ on MIPₚᵢ and MIPₜᵢ were explained as follows: the retentions of PZ, CPZ and PMZ were maximal at apparent pH 8.2 of a mobile phase (apparent pKₐ value of PZ, CPZ or PTZ in the mobile phase), those were decreased with a decrease of mobile phase pH from 8.2 because of suppression of dissociation of the imprinted MAA-co-EDMA polymers and those were decreased with an increase of mobile phase pH from 8.2 because of suppression of protonation of PZ, CPZ and PMZ. Since there was no dissociation group in PTZ, its retention remained almost unchanged.

Fig. 4 shows the effects of acetonitrile contents on the retentions of PZ, CPZ, PMZ and PTZ on MIPₜᵢ, where water / acetonitrile including 5 mmol L⁻¹ ammonium formate was used as the mobile phase. The retentions of all solutes were decreased with an increase of the
acetonitrile contents from 70 to 90 vol%, while at 95 vol% acetonitrile content the retentions of PZ, CPZ and PMZ were increased compared with those at 90 vol% acetonitrile content. On the other hands, the retention of PTZ was decreased with an increase of acetonitrile contents. These results indicate that in low acetonitrile contents hydrophobic interactions work for the retentions of PZ, CPZ and PMZ, and that hydrophilic interactions seem to work in high acetonitrile contents. On the other hands, only hydrophobic interactions seem to work for the retention of PTZ on MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}. In conclusion, ionic and hydrophobic interactions seem to work for the retentions of PZ, CPZ and PMZ shown in Fig. 3A and B, while only hydrophobic interactions seem to work for those of PTZ.

**Evaluation of molecular-recognition abilities of MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}**

Fig. 5 shows the IFs of PZ, CPZ, PMZ and PTZ on MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}, where 50 mmol L\textsuperscript{-1} potassium phosphate buffer / acetonitrile (30/70, v/v) was used as the mobile phase. The maximal IFs of PZ, CPZ and PMZ on the respective MIPs were obtained at apparent pH 7 of a mobile phase. On MIP\textsubscript{PZ}, the IFs of PZ, CPZ and PMZ were 7.4, 6.9 and 3.6, respectively, while on MIP\textsubscript{CPZ}, those were 15.2, 20.6 and 7.6, respectively. However, PTZ was not recognized on MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}. As mentioned above, the template molecules (PZ and CPZ, respectively) were recognized the most on the respective MIPs (MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}). This means that in addition to shape recognition, ionic and hydrophobic interactions seem to work for recognition of PZ and CPZ on the respective MIPs. Furthermore, note that the IF of PZ was higher on MIP\textsubscript{CPZ} than MIP\textsubscript{PZ}, 15.2 on MIP\textsubscript{CPZ} and 7.4 on MIP\textsubscript{PZ}. MIP\textsubscript{WF} and MIP\textsubscript{CWF}, which are prepared using 4-VPY and EDMA as the functional monomer and crosslinker, respectively, by multi-step swelling and polymerization, showed the similar phenomena with MIP\textsubscript{PZ} and MIP\textsubscript{CPZ}: the IF of WF was larger on MIP\textsubscript{CWF} than MIP\textsubscript{WF}. More hydrophobic imprinting sites for MIP\textsubscript{CPZ} were formed compared with those for MIP\textsubscript{PZ}; that is, PZ was more retained on MIP\textsubscript{CPZ} than MIP\textsubscript{PZ} as
shown in Fig. 3. Further studies why the retention and imprinting factors of PZ are higher on MIP_{CPZ} than MIP_{PZ} are on-going in our laboratory.

**Selective extraction of PZ in rat serum samples**

It is well known that the leakage of a trace amount of the template molecule from the resultant MIP affects on the accuracy and precision of its analysis.\textsuperscript{22-25} MIP_{CPZ} is suitable for the selective extraction of PZ for avoiding the leakage problems. Furthermore, the above mentioned results indicate that PZ was retained and recognized more strongly on MIP_{CPZ} than MIP_{PZ}. We used MIP_{CPZ} as a pretreatment column for selective extraction of PZ in rat serum samples and an ODS column as an analytical column in column-switching LC with fluorescence detection. However, PZ did not elute from MIP_{CPZ} completely due to strong interactions of PZ with MIP_{CPZ}. Thus, we tried to add CPZ to serum samples to recover PZ from MIP_{CPZ} completely. As shown in Table 1, the recoveries of PZ from rat serum sample was increased with an increase of a CPZ concentration added in rat serum samples. Those were almost constant by addition of 20 µg mL\textsuperscript{-1} of CPZ or more as the final concentration. In the following experiments, 20 µg mL\textsuperscript{-1} of CPZ was added to rat serum samples.

Fig. 6A shows a chromatogram of rat serum samples without addition of CPZ using MIP_{CPZ} as the pretreatment column in column-switching LC, where a mixture of ammonium formate and acetonitrile for pretreatment, and a mixture of formic acid and acetonitrile for analysis were used as the mobile phases. Almost no peaks were observed on the chromatogram. Fig. 6B and C shows chromatograms of rat serum samples spiked with 1.0 µg mL\textsuperscript{-1} of PZ with addition of 20 µg mL\textsuperscript{-1} of CPZ using MIP_{CPZ} and NIP, respectively, as the pretreatment columns in column-switching LC. As shown in Fig. 6B, PZ was completely recovered and CPZ added was also eluted. On the other hands, PZ was not retained on NIP but CPZ was partially retained. Thus, PZ was not observed but CPZ was eluted on a chromatogram (Fig. 6C). MIP_{CPZ} could be
tolerable for 500 injections or more of deproteinized rat serum samples.

Table 2 shows the intra- and inter-day accuracy and precision data for the assays of PZ in rat serum samples. The calibration graph, constructed from peak area of PZ, was linear with a coefficient of determination of $> 0.9999$ in the concentration ranges of $0.02–20.0 \, \mu\text{g mL}^{-1}$ with a 100 \, \mu\text{L injection of deproteinized rat serum samples, and the equation was } y = 370381x + 5123.7 \text{ for PZ, where } x \text{ is the PZ concentration and } y \text{ is the peak area. The limits of quantitation and detection of PZ were 0.02 \, \mu\text{g mL}^{-1} \text{ and } 0.005 \, \mu\text{g mL}^{-1} \text{ at a signal to noise ratio of 5, respectively.}

Conclusions

Monodisperse \text{MIP}_{\text{PZ}} \text{ and MIP}_{\text{CPZ}} \text{ were prepared by multi-step swelling and polymerization using MAA as a functional monomer and EDMA as a crosslinker. The retention and molecular-recognition properties for PZ and CPZ were evaluated using a mixture of potassium phosphate buffer and acetonitrile, or a mixture of ammonium formate and acetonitrile as the mobile phase in LC. PZ and CPZ gave the maximal retentions on \text{MIP}_{\text{PZ}} \text{ and MIP}_{\text{CPZ}} \text{ at apparent pH 8.2 of a mobile phase using a mixture of potassium phosphate buffer and acetonitrile as the mobile phase. The retentions of PZ and CPZ were decreased with an increase of acetonitrile contents from 70 to 90 vol\% using a mixture of ammonium formate and acetonitrile as the mobile phase. The template molecules (PZ and CPZ, respectively) were recognized the most on the respective MIPs (\text{MIP}_{\text{PZ}} \text{ and MIP}_{\text{CPZ}}). These results indicate that in addition to shape recognition, ionic and hydrophobic interactions seem to work for the retention and molecular-recognition of PZ and CPZ on the MIPs. It is interesting that the IF of PZ was higher on \text{MIP}_{\text{CPZ}} \text{ than \text{MIP}_{\text{PZ}}, 15.2 on \text{MIP}_{\text{CPZ}} and 7.4 on \text{MIP}_{\text{PZ}}. This result indicates that the use of \text{MIP}_{\text{CPZ}} \text{ instead of \text{MIP}_{\text{PZ}} is suitable for the recognition of PZ and for avoiding the leakage}}
problems in the assay of PZ in column-switching LC. The addition of CPZ to serum samples was required to recover PZ from MIP_{CPZ} completely because of strong interactions of PZ with MIP_{CPZ}. MIP_{CPZ} was successfully utilized for selective extraction of PZ in rat serum samples in column-switching LC with fluorescence detection.

Acknowledgements

This work was supported in part by JSPS KAKENHI Grant Number JP16K08212 to JH.

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Table 1  Effect of CPZ concentration on recovery of PZ in rat serum samples

| CPZ concentration (μg/mL) | 0      | 5      | 10     | 20     | 40     | 100    |
|--------------------------|--------|--------|--------|--------|--------|--------|
| Recovery (%) ± RSD\(b\)  | 87.1 ± 0.54 | 96.8 ± 0.46 | 97.7 ± 0.37 | 98.4 ± 0.16 | 98.6 ± 0.19 | 96.9 ± 0.47 |

\(a\) The following column-switching LC conditions are used. Pretreatment conditions: pretreatment column, MIP\(_{CPZ}\) (10 mm × 4.0 mm i.d.); column temperature, 30 °C; mobile phase, 50 mmol L\(^{-1}\) ammonium formate / acetonitrile (20/80, v/v); flow rate, 0.8 mL min\(^{-1}\); injection volume, 100 μL. Analysis conditions: analytical column, Cosmosil 5C\(_{18}\) -AR-II (150 mm × 4.6 mm i.d.); column temperature, 30 °C; mobile phase, water / acetonitrile (70/30, v/v) including 0.5 vol% formic acid; flow rate, 0.8 mL min\(^{-1}\); detection, excitation and emission wavelengths at 315 and 448 nm, respectively.

\(b\) Rat serum samples spiked with 1.0 μg mL\(^{-1}\) of PZ with addition of different concentrations of CPZ, to which the same volume of acetonitrile was added, deproteinized and centrifuged. Recovery (%) = (Peak area of PZ in rat serum samples in column-switching LC)/(Peak area of PZ in water samples injected onto an analytical column directly) × 100
Table 2  Intra-day and Inter-day precision and accuracy data for the assays of PZ spiked in rat serum samples.

| Analyte | Intra-day (n=5) | Inter-day (n=15) |
|---------|----------------|-----------------|
|         | Nominal concentration | Mean | Accuracy | RSD | Mean | Accuracy | RSD |
|         | (μg mL⁻¹) | (μg mL⁻¹) | (%) | (%) | (μg mL⁻¹) | (%) | (%) |
| PZ      | 0.020 | 0.021 | 107.0 | 2.1 | 0.019 | 97.3 | 4.4 |
|         | 0.50 | 0.46 | 92.9 | 0.90 | 0.46 | 92.9 | 1.4 |
|         | 5.00 | 5.12 | 102.4 | 0.40 | 5.17 | 103.4 | 1.5 |
|         | 20.0 | 20.2 | 101.1 | 0.30 | 20.7 | 103.5 | 2.4 |

Column-switching LC conditions as in Table 1.
Figure Captions

Fig. 1 Structures of PZ, CPZ, PMZ and PTZ used in this study.
log P and pK_a from ref. 31.

Fig. 2 SEM images of (A) MIP_{CPZ}, (B) MIP_{CPZ} and (C) NIP.

Fig. 3 Effect of mobile phase pH on the retention factors of PZ, CPZ, PMZ and PTZ on (A) MIP_{PZ} and (B) MIP_{CPZ}.
LC conditions: column size, 10 mm × 4.6 mm i.d.; mobile phase, 50 mmol L^{-1} potassium phosphate buffer / acetonitrile (30/70, v/v); column temperature, 25 ℃; flow rate, 0.3 mL min^{-1}; detection, 255 nm; loaded amount, 2 μg. Keys: △, PZ; ○, CPZ; □, PMZ; ◇, PTZ.

Fig. 4 Effect of acetonitrile content on the retention factors of PZ, CPZ, PMZ and PTZ on MIP_{CPZ}.
LC conditions: column size, 10 mm × 4.6 mm i.d.; mobile phase, water / acetonitrile including 5 mmol L^{-1} ammonium formate; column temperature, 25 ℃; flow rate, 0.3 mL min^{-1}; detection, 255 nm; loaded amount, 2 μg. Keys as in Fig. 3.

Fig. 5 Effect of mobile phase pH on the imprinting factors of PTZ, PMZ, PZ and CPZ on (A) MIP_{PZ} and (B) MIP_{CPZ}.
LC conditions and keys as in Fig. 3.

Fig. 6 Chromatograms of (A) rat serum samples (without addition of CPZ) using MIP_{CPZ} as a pretreatment column and those spiked with PZ (1.0 μg mL^{-1}) with addition of CPZ (20 μg mL^{-1})
using (B) MIP<sub>CPZ</sub> and (C) NIP as a pretreatment column in column-switching LC.

Column-switching LC conditions as in Table 1 except that pretreatment columns used are MIP<sub>CPZ</sub> and NIP (10 mm × 4.0 mm i.d.).
Fig. 1 Temperature dependence of the concentration of water saturated in pure nitrobenzene.

Promazine (PZ)
$\log P = 4.69, \ pK_a = 9.43$

Chlorpromazine (CPZ)
$\log P = 5.18, \ pK_a = 9.41$

Promethazine (PMZ)
$\log P = 4.89, \ pK_a = 8.98$

Phenothiazine (PTZ)
$\log P = 4.15$
Fig. 2  SEM images of (A) MIP<sub>CPZ</sub>, (B) MIP<sub>CPZ</sub> and (C) NIP.
Fig. 3  Effect of mobile phase pH on the retention factors of PZ, CPZ, PMZ and PTZ on (A) MIP\textsubscript{PZ} and (B) MIP\textsubscript{CPZ}.

LC conditions: column size, 10 mm × 4.6 mm i.d.; mobile phase, 50 mmol L\(^{-1}\) potassium phosphate buffer / acetonitrile (30/70, v/v); column temperature, 25 °C; flow rate, 0.3 mL min\(^{-1}\); detection, 255 nm; loaded amount, 2 μg. Keys: △, PZ; ○, CPZ; □, PMZ; ◇, PTZ.
Fig. 4  Effect of acetonitrile content on the retention factors of PZ, CPZ, PMZ and PTZ on MIP<sub>CPZ</sub>.

LC conditions: column size, 10 mm × 4.6 mm i.d.; mobile phase, water / acetonitrile including 5 mmol L<sup>-1</sup> ammonium formate; column temperature, 25 °C; flow rate, 0.3 mL min<sup>-1</sup>; detection, 255 nm; loaded amount, 2 μg. Keys as in Fig. 3.
Fig. 5  Effect of mobile phase pH on the imprinting factors of PTZ, PMZ, PZ and CPZ on (A) MIP\textsubscript{PZ} and (B) MIP\textsubscript{CPZ}.

LC conditions and keys as in Fig. 3.
Fig. 6 Chromatograms of (A) rat serum samples (without addition of CPZ) using MIP_CpZ as a pretreatment column and those spiked with PZ (1.0 μg mL⁻¹) with addition of CPZ (20 μg mL⁻¹) using (B) MIP_CpZ and (C) NIP as a pretreatment column in column-switching LC. Column-switching LC conditions as in Table 1 except that pretreatment columns used are MIP_CpZ and NIP (10 mm × 4.0 mm i.d.).
Graphical Index

- **MIP<sub>CPZ</sub>**
  - Time (min): 0 to 30
  - Peaks: CPZ, PZ

- **NIP**
  - Time (min): 0 to 30
  - Peaks: CPZ

**Selective extraction of PZ in rat serum**

**No extraction of PZ in rat serum**