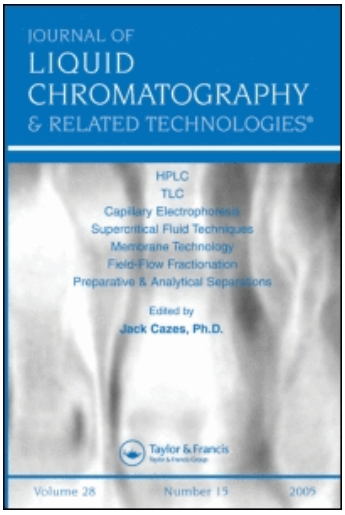


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# PREPARATION OF MOLECULAR IMPRINTED MONOLITHIC EXTRACTION COLUMN AND ITS APPLICATION TO STRYCHNINE ANALYSIS IN WAN TONG JIN GU TROCHE BY HPLC

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# PREPARATION OF MOLECULAR IMPRINTED MONOLITHIC EXTRACTION COLUMN AND ITS APPLICATION TO STRYCHNINE ANALYSIS IN WAN TONG JIN GU TROCHE BY HPLC

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□ *The preparation and performance of a molecular imprinted monolithic extraction column (MIMEC) for strychnine analysis are described. The MIMEC was prepared by in-situ copolymerization of Strychnine, methacrylic acid, and ethylene glycol dimethacrylate in a methacryloyl-activated glass tube. The effect of eluent composition on strychnine recovery was investigated and the optimized enrichment and washing conditions were obtained. The column was used to enrich the strychnine from a Wan Tong Jin Gu troche. The results show that the strychnine-imprinted monolithic extraction column could effectively enrich and purify strychnine from samples.*

**Keywords** enrich, HPLC, in-situ, molecular imprinted, monolithic extraction column, strychnine

## INTRODUCTION

Sample pretreatment, including reduction of impurities and enrichment of trace analytes, plays an important role in the analysis of trace and ultra-trace substances in complex matrices such as biological materials, drugs, and environmental samples.[1]

The development of sample pretreatment technology has been slow in comparison with analytical instrument development. Traditional sample pretreatment methods are often labor-intensive and time-consuming. Macroporous polymer and silica packed columns, developed in the 1970s, provided new methods for pre-separation and pre-enrichment of samples, owing to the advantages of lower solvent consumption, lower

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sample contamination, shorter pretreatment times, and various forms of solid phase extraction technology. At present, the most used solid phase extraction (SPE) sorbents are alumina, silica, active carbon, $C_{18}$, and ion exchange resins. However, the specificity of these sorbents is low. As a result, many matrix components and contaminants are also enriched along with the analyte, which interferes with the subsequent analysis.[3] Immune-adsorbents with high specificity, based on antibodies, have been developed in recent years, but their preparations are complicated and time-consuming. Furthermore, there are always a few types of antibody commercially available. These adsorbents also typically suffer from poor mechanical strength and stability.[4]

The use of molecularly imprinted polymers (MIPs) as sorbents for solid phase extraction can overcome the disadvantages of conventional adsorbents due to their high selectivity and affinity.[5] Compared with immune-adsorbents, MIPs sorbents provide much longer lifetime because of their high stability. Since its first introduction in 1994,[6] molecular imprinted polymers have been widely used for enrichment and separation of low concentration substances in complex samples.[7,8] Mostly, imprinted polymers are prepared by bulk polymerization, followed by crushing, grinding, and sieving to provide molecularly imprinted polymer particles for various applications. These procedures are tedious and time-consuming. Furthermore, the resulting particles are irregular in size and shape and some interaction sites are destroyed during grinding, which leads to a negative impact on recognition performance and lower loading capacity. In this study, a molecular imprinted monolithic column with good selectivity for strychnine was prepared by in-situ polymerization in a methacryloyl-activated glass tube. The extraction column was characterized by easy preparation, low backpressure, and was used to enrich strychnine from a Wan Tong Jin Gu troche.



## EXPERIMENTAL

### Materials and Instruments

Methacrylic acid (MAA) was purchased from Tianjin Chemical Reagent Plant (Tianjin, China). Ethylene glycol dimethacrylate (EDMA) and 3-(triethoxysilyl) propyl methacrylate were obtained from Aldrich (Milwaukee, USA). Strychnine was purchased from Shanghai No. 2 Chemical Reagent Factory (Shanghai, China). Brucine was purchased from Fluka (Switzerland). 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent Factory (Shanghai, China). Acetonitrile was HPLC grade. All the other reagents were analytical grade. MAA was distilled under vacuum to remove any inhibitors before polymerization. EDMA

was purified by extraction with 10% aqueous sodium hydroxide and water and dried over anhydrous magnesium sulfate before use.

### Preparation of the Molecular Imprinted Monolith Polymer Extraction Column Pretreatment of the Glass Tube

The glass tube was first filled with 1.0 mol/L NaOH for 2 h, washed with water and filled with 2.0–3.0 mol/L HCl for 2 h, and then washed again with water until the eluent was neutral. The glass tube was dried at 160°C for 2–3 h to remove the residual water. Five percent (v/v) 3-(triethoxysilyl) propyl methacrylate in toluene was freshly prepared and filled into the glass tube, purged with nitrogen, and refluxed for 10 h at a temperature between 90 and 95°C. The residual solution was then driven out and the glass washed thoroughly with toluene. Finally, the glass tube was dried with nitrogen.[9]



### Preparation of Molecular Imprinted Polymer for Strychnine

Template strychnine (25.5 mg), monomer MAA (0.025 mL), EDMA (0.22 mL), toluene (0.77 mL) and dodecanol (0.39 mL) were mixed and degassed by ultrasonication for 10 min. AIBN (2.4 mg) was added and the mixture was transferred to the glass tube. Both ends of the glass tube were sealed with a septum, and the tube was placed in an oven at 50°C for 12 h. The monolith column was then washed with methanol and acetic acid (4:1, v/v) until no strychnine was detected in the eluent. A blank column was prepared by the same method, but without the strychnine template.[10]

### Instrumental and Analytical Conditions

HPLC analysis was performed on an Agilent 1100 system consisting of an LC-10Advp pump and SPD-10Avp UV-Vis Spectra spectrophotometer (USA). The data were acquired and processed with Empower software. HPLC separations were performed at a flow-rate of 0.50 mL $min^{-1}$ or 1.0 mL $min^{-1}$, at 25°C. Spectrometric detection was conducted at 254 nm. Standards (1 mg $mL^{-1}$ in ethanol, 10 μL sample volume) were injected onto the $C_{18}$ column (Eclipse mesylate).

### Extraction Procedures

Prior to sample loading, the column was washed with 0.50 mL of disodium hydrogen phosphate buffer and methanol solution (70%, pH 7.0) three times. The sample was then injected, followed by washing with

0.50 mL of disodium hydrogenphosphate buffer and methanol solution (70%, pH 7.0) three times. Desorption was conducted by washing the MIPMEC with disodium hydrogen phosphate buffer and methanol solution (50%, pH 2.0).

### Sample Preparation

Five pieces of sample troches were ground and 1.0 g of the powder was dissolved in 3.0 mL 0.10 mol/L NaOH and adjusts to pH 9.0 with HCl. The solution was incubated at room temperature for 30 min. Then, 20.0 mL of chloroform were added to sample solution three times, the supernatant was collected and dried under vacuum, then the dry extract was dissolved with 2.0 mL of methanol; finally, the solution was subjected to extraction process as described above. The solutions obtained before and after elution were determined by HPLC.



## RESULTS AND DISCUSSION

### Optimization of Enrichment Conditions

Elution and enriching conditions were studied to achieve good recovery. The extraction recovery of the strychnine was calculated by comparing the extracted amounts of strychnine from sample with the total amounts loaded. 0.50 mg of strychnine (1.0 mg/mL) was loaded on the monolithic extraction column, and the adsorbed sample was then eluted with 0.50 mL of methanol-water solution containing various amounts of methanol. As shown in Fig. 1, the maximum recovery of strychnine was obtained

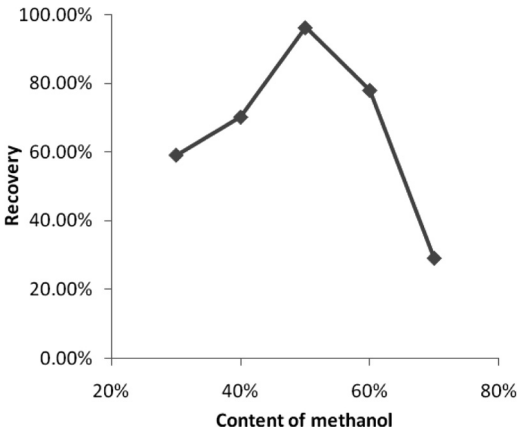


**FIGURE 1** Effect of methanol concentration on the recovery of strychnine.

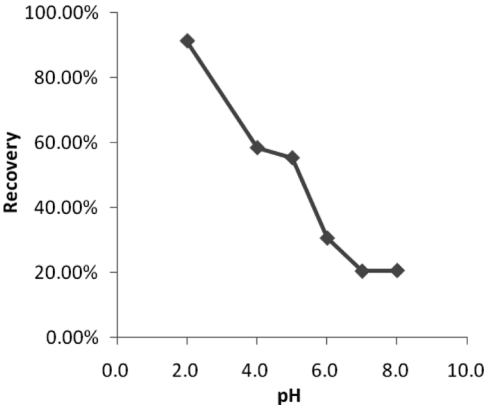


**FIGURE 2** Recovery using different pH buffers with a methanol ratio of 1:1.



by using 50% (v/v) aqueous-methanol solution as eluent. The minimum recovery occurred at an aqueous-methanol solution concentration of 70% (v/v). This suggests that strychnine adsorbed on the MIP column could be eluted when the content of methanol was 50%. Strychnine could be retained on the MIP column when the methanol content was 70%.

The influence of eluant pH on strychnine recovery was also studied, using disodium hydrogen phosphate buffer and methanol solution (1:1). It can be seen from Fig. 2 that the maximum recovery of strychnine occurred at pH 2.0 and the minimum recovery occurred at pH 7.0.

According to the above results, disodium hydrogen phosphate buffer and methanol solution (70%, pH 7.0) was chosen as the cleaning and enrichment solution, while disodium hydrogenphosphate buffer and methanol solution (50%, pH 2.0) was chosen as the elution solution.

## Determination of Recovery

To validate the reliability of aqueous 50% methanol solutions for elution and to demonstrate the reproducibility of the MIMEC, different concentrations of strychnine solution were applied. It can be seen in Table 1

**TABLE 1** Recoveries of Strychnine on MIP by Loading Aqueous Solutions with Different Concentrations ($n = 3$)

| Sample | Loaded (mg) | Detected (mg) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 0.0500 | 0.0436 | 87.3 | 1.9 |
| 2 | 0.100 | 0.0896 | 89.6 | 2.2 |
| 3 | 0.150 | 0.1300 | 86.7 | 2.9 |

**TABLE 2** Recoveries of Strychnine on NIP by Loading Aqueous Solutions with Different Concentrations ($n = 3$)

| Sample | Loaded (mg) | Detected (mg) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 0.0500 | 0.0266 | 53.2 | 3.1 |
| 2 | 0.100 | 0.0514 | 51.4 | 3.5 |
| 3 | 0.150 | 0.0808 | 53.9 | 4.2 |

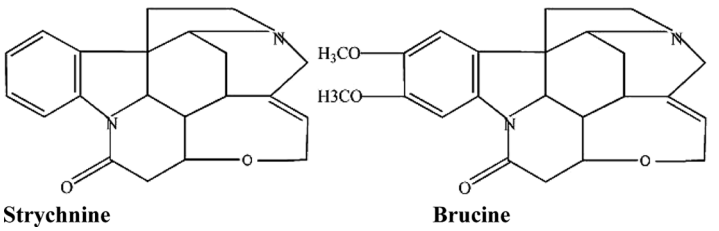


**FIGURE 3** Structures of strychnine and brucine.



that the recoveries of strychnine on the MIMEC were >85%. The retention behavior and recoveries in the non-imprinted column were also determined for comparison (Table 2). It is obvious that the MIMEC has the good recoveries and enriching ability for strychnine. The precisions of recovery were calculated as relative standard deviation (RSD), showing values lower than 2.9 and 4.2%, respectively.

## Determination of the Recovery of a Structural Analogue

The separation of structurally similar compounds required not only a highly specific MIP extraction column but also optimum elution conditions. Brucine, which is a structural analogue of strychnine (as shown in Figure 3), was used to evaluate the specificity of the MIMEC. The loading, washing, and eluting of brucine on the MIMEC was performed under the optimum conditions for strychnine. Table 3 summarizes the recovery of brucine on MIMPEC at various loading amounts. We can see the method developed in this paper has higher selectivity for strychnine.

**TABLE 3** Recoveries of Brucine on MIP by Loading in Aqueous Solution at Different Concentrations ($n = 3$)

| | Loaded (mg) | Detected (mg) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 0.0500 | 0.0250 | 40.0 | 2.5 |
| 2 | 0.100 | 0.0343 | 34.3 | 1.7 |
| 3 | 0.150 | 0.0517 | 34.5 | 2.4 |

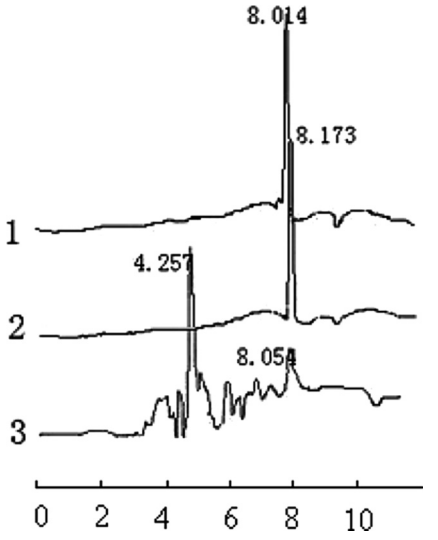


**FIGURE 4** Chromatogram of strychnine in a Wan Tong Jin Gu Troche.



### Determination of Strychnine in a Real Sample

A 0.50 mL sample solution was loaded on to the MIMEC, and then sequentially washed with pH 7.0 buffer containing 70% methanol and pH 2.0 buffer containing 50% methanol. The solutions obtained before and after elution were determined by HPLC.

Eclipse mesylate-$C_{18}$ column, mobile phase: methanol-acetonitrile-$KH_2PO_4$ (10 mmol/L) triethylamine ($H_3PO_4$ adjusted to pH 2.5) (Volume 5:10:85:0.5); flow rate: 0.50 mL/min; detection wavelength: 254 nm; temperature of 30°C.

Trace 1 shows the chromatogram of the strychnine standard, trace 2 shows the chromatogram of after the extraction by MIMEC, and trace 3 was chromatogram of before extraction by MIMEC. From Fig. 4, we can clearly see that the MIP column could effectively enrich and purify the strychnine from the sample.

## CONCLUSION

A new MIMEC method was developed and validated for extraction and enriching strychnine from pharmaceutical sample. The MIMEC was designed and the optimum elution conditions were obtained. As an example, the strychnine contained in a Wan Tong Jin Gu troche (used to cure waist and leg aches) was enriched and purified. The results demonstrated that MIPMEC could enrich and purify the target molecule

effectively under normal pressure. Given the simplicity, low cost, speed, and satisfactory results achieved in this study, it is expected that this technique can be extended to other analytes and will provide a novel method for pretreatment of samples.

## ACKNOWLEDGMENTS

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