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Preparation and Evaluation of Molecularly Imprinted Monolithic Column for Felodipine in Micro-liquid Chromatography

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Abstract: This study concentrated on the production of molecularly imprinted polymers (MIPs) as highly selective sorbents for felodipine (FLD), a representive dihydropyridine calcium antagonists. Demonstrated chromatographically through a selection factor, these MIPs showed high selectivity for the template molecule among a group of structurally similar compounds. The recognition was found to correlate with structural similarity to the template compound.

Keywords: Molecularly Imprinted polymers, felodipine.

Molecularly imprinted polymers (MIPs) are synthetic ones that can be made to exhibit spatial ''memory'' of a template molecule that is incorporated *prior* to polymerization[1-4]. During the last years, applications of the materials as affinity phases in solid-phase extractions[5], as recognition elements in sensors[6], as stationary phase for preparative purification, as catalysts, or as adsorbents for therapeutic use are being actively pursued. Depending upon the nature of the chemical bonds involved, the techniques of MIP synthesis can be classified into two main categories: (a) covalent approach, and (b) non-covalent approach. In the covalent approach, a template–monomer complex is formed through reversible covalent bonds, whereas in the non-covalent approach, the monomer–template complex is formed by non-covalent interactions such as hydrogen bonding, electrostatic, and hydrophobic interactions[7–9]. In order to remove the template, the covalent bonds connecting the template to the polymer should be cleaved. On the other hand, non-covalent imprinting has been reported to be a more direct and flexible approach because it can be imprinted in larger range of compounds. However, it is hard to find a suitable system for every template, where the binding is strong enough to make acceptable cavities yet easily cleavable to achieve fast and easy extraction of the template from the network.

Felodipine is a selective calcium channel antagonist of the dihydropyridine class

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used in the treatment of hypertension. To date, there is no report on the preparation of a MIP column and its performance in micro-liquid chromatography. Here, FLD is selected as the template, MIP monolithic columns were evaluated for the performance of MIP monolithic columns.

Fused-silica capillaries of 100 μm I.D. and 375 μm O.D. were purchased from Hebei Yongnian Optical Fiber Factory (Yongnian, Hebei, China). The chemical reagents were purchased from the sources indicated in parentheses: 3-(trimethoxysilyl) propyl methacrylate (Sigma-Aldrich, St. Louis, MO, USA); methacrylic acid (MAA) (Beijing Donghuan Chemical Reagent Factory, Beijing, China); ethylene glycol dimethacrylate (EGDMA) (Suzhou Anli Chemical & Engineering Co. Ltd., Suzhou, China); MAA and EGDMA were distilled in vacuum and collected over type 4Å molecular sevies. 2, 2-Azobis (2-isobutyronitrile) (AIBN), (Special Chemical Reagent Factory, Nankai University, Tianjin, China), which was recrystallized from ethanol before use. Nicadipine (NCD), nisoldipine (NSD) and FLD employed in the study were obtained from Tianjin Institute of Pharmaceutical Control, (Tianjin, China). Other analytical reagents such as thiourea were purchased from Tianjin Chemical Reagent Co. Lt. (Tianjin, China). Phosphate and acetonitrile were of HPLC grade. All solvents and solutions for pCEC analysis were filtered through a 0.22 μm cellulose ester membrance.

A fused-silica capillary was silanized with 3-(trimethoxysilyl)propyl methacrylate, according to the procedure published by Hjérten[10]. A mixture of FLD (0.08 mmol), MAA (0.12 mmol), EGDMA (0.50 mmol) and initiator AIBN were dissolved in proper volume of toluene. The mixture was degassed for 3 min by ultrasonication. A 40 cm length capillary was attached to a syringe and filled with the degassed polymerization mixture to a length of 25 cm. After each end of the column was plugged with a piece of rubber, the capillary was submerged in a 60℃ bath for 24 h. Subsequently, the column was moved out of the water bath and immediately washed with 5% acetic acid in acetonitrile in order to wash the imprint molecules, the unreacted monomers and the remainder of the initiator out of the capillary column. At last, the column was washed with acetonitrile. A detection window was created near the end of the continuous polymer bed by burning off a 2 mm segment of the polyimide outer coating. The final capillary column had a total length of 35 cm and the effective length with MIP-based stationary phase was 20 cm, which was then stored at room temperature until use. A non-imprinted polymer (NIP) monolithic column (in the absence of template) was prepared and treated in an identical manner.

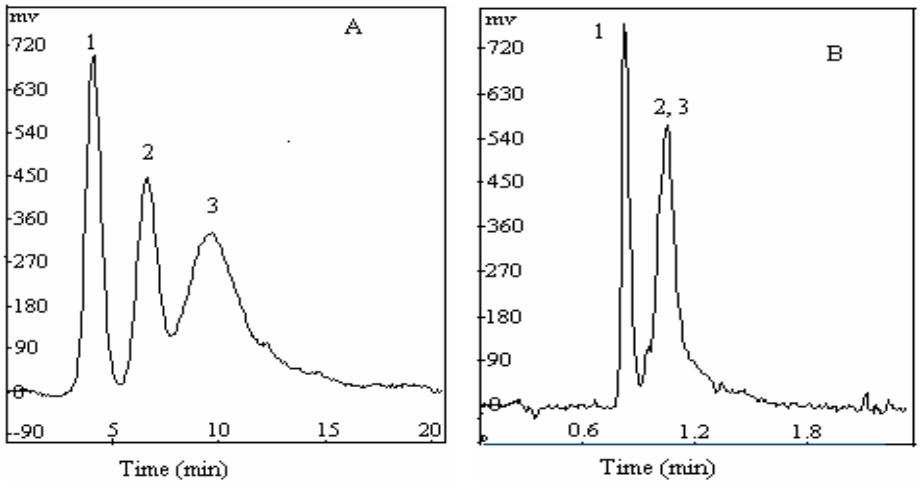
In these studies, experiments were carried out on a Trisep^{TM} 2010GV system (Unimicro Technologies, Pleasanton, CA, USA), which comprised an intelligent HPLC pump, a variable-wavelength UV–Vis detector, a micro fluid manipulation module (including a 10 nL four-port injector) and a data acquisition module. The samples were introduced through the rotary injector.

For a given compound, the ratio of its retention factors on the MIP and NIP columns (K'_{MIP}/ K'_{NIP}) is of interest, as it provides the selectivity of MIP for the compounds. This ratio, for which the term selection factor will be introduced, would be expected to be highest for the template molecule. The selection factors for compounds

are useful in comparing their relative affinities for the MIP, which are 3.51, 6.18 and 7.96 for NCD, NSD and FLD, respectively. Upon inspection of these data, some trends were observed. FLD and NSD exhibited greater affinity for the MIP than NCD. This behavior is not surprised in view of the fact that FLD has a similar shape and space steric hinderance with NSD. The separation of FLD, NSD and NCD on MIP monolithic column and NIP column are shown in **Figure 1**. FLD and NSD can not be separated on the blank column (**Figure 1-B**). On MIP monolith, the retention times of FLD, NSD and NCD is 8.289, 6.432 and 3.085 min, respectively (**Figure 1-A**). In our experiments, we found that the MIP column has more flow resistance than NIP column has. This result was in disagreement with publish paper[11].

In summary, FLD was well separated on a MIP-imprinted monolithic capillary in HPLC mode. NSA also exhibited strong affinity to MIP. The procedure of fabrication for capillary column is simple and low consumption of chemicals meets the demands of green chemistry.

Figure 1 The chromatogram of FLD (3), NSD (2) and NCD (1) on MIP column (A) and NIP column (B).



Experimental conditions: mobile phase: 60 % (v/v) acetonitrile in 30 mmol/L phosphate (pH 5.4); flow rate 0.02 mL/min; detection wavelength 254 nm; injection volume, 10 nL.

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