Chinese Chemical Letters Vol. 11, No. 1, pp. 65–68, 2000

Simultaneous Chiral Separation Using a Combinatorial Molecular Imprinting Phase

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Abstract: Molecular imprinting chiral stationary phase against Cbz-L-Serine (Cbz-L-Ser) and Cbz-L-Alaine (Cbz-L-Ala) were prepared utilizing acrylamide + 2-vinylpyridine as combined basic functional monomers. Cross-selectivity was used to obtain simultaneous chiral separations of Cbz-DL-Ser and Cbz-DL-Ala by connecting two columns packed with Cbz-L-Ser and Cbz-L-Ala imprinted chiral stationary phase, respectively.

Keywords: Molecular imprinting polymer, chiral stationary phase, chiral separation.

Introduction

Molecular imprinting is a technology by which specific recognition sites can be produced by using a template molecule in the polymerization procedure. In recent years, molecular imprinting has become an important approach for the preparation of chiral stationary phase with predetermined selectivity[1-3]. So far, the commonly utilized functional monomers include methacrylic acid[2], acrylamide[4] and 4-vinylpyridine[5], combined functional monomers such as methacrylic acid + 2-vinylpyridine[6-7] and acrylamide + 2-vinylpyridine[8] were also introduced to improve the imprinting efficiency. It has been commonly found that not only the template enantiomer but also those with the structure similar to the template can be separated by the molecular imprinting chiral stationary phase. This is called cross-selectivity. Recently, a combinatorial stationary phase was evaluated using the cross-selectivity properties of the molecular imprinting chiral phase[9]. Simultaneous separation of several compounds was achieved by the combinatorial stationary phase.

In this communication, we demonstrated that the combinatorial stationary phase could be obtained by connecting the columns packed with Cbz-L-Ser and Cbz-L-Ala imprinted chiral stationary phases, respectively. Simultaneous chiral separations of Cbz-DL-Ser and Cbz-DL-Ala were achieved on this combinatorial stationary phase.

Experimental

Material

Cbz-D-Ala, Cbz-L-Ala, Cbz-D-Ser and Cbz-L-Ser were purchased from Sigma (St. Louis, MO, USA) and 2-VP from Acros (Pittsburgh, PA, USA). Ethylene glycol

dimethacrylate (EDMA) was obtained from Shanhu Chemical Plant (Shanghai), Azo-bis-isobutyronitrile (AIBN) and acrylmide (AM) were from Beijing Chemical Plant (Beijing). Inhibitors in EDMA and 2-VP were removed by active carbon. All the solvents were HPLC or analytical grade.

Preparation of molecular imprinting polymers

Molecular imprinting polymers P1 and P2 against Cbz-L-Ala and Cbz-L-Ser were prepared by photo initiation as described in reference 8. The molar ratio of Cbz-L-Ala or Cbz-L-Ser to acrylamide, 2-vinylpyridine and ethylene glycol dimethacrylate was 1:2:2:20. The bulk polymer was grounded, sieved and sedimented, particles with diameters smaller than 35 μm were collected.

Chromatography

The collected molecular imprinting particles were slurried and packed into 250×4mm columns at 10 MPa. Chromatography was carried out using a LC-890A system from Beijing Xingda Technology Development Company (Beijing) consisting of two LP-05C pumps equipped with a LC-830 UV-VIS detector (Soma Optic LTD, Japan). The samples were injected through a Rheodyne 7125 valve loop injector, and monitored at 254 nm. The system was controlled by a JS-3030 Chromatography Operation Station (Jiangshen Separation Technology Company, Dalian). Acetone was used as void marker. Enantio-selectivity (α) were calculated according to standard chromatographic theory. Typical for the molecular imprinting chiral stationary phase, the chromatographic peak obtained was asymmetric because of the heterogeneity of the recognition site, so resolution was expressed as the resolution function (f/g) according to reference [10].

Result and disscusion

The chiral recognition ability of the molecular imprinting polymers was evaluated in HPLC-mode. It has been demonstrated that combined functional monomer acrylamide + 2-vinylpyridine were suitable for the imprinting of amino acid derivatives[8]. As expected, excellent chiral recognition ability of molecular imprinting polymers against Cbz-L-Ala and Cbz-L-Ser were achieved and optimal selectivity for Cbz-DL-Ala and Cbz-DL-Ser were observed on Cbz-L-Ala and Cbz-L-Ser imprinted chiral stationary phases P1 and P2, respectively (**Table 1**). Cross-selectivity results of P1 and P2 were also shown in **Table1**.

Simultaneous separation of several compounds on one stationary phase is valuable, for example, in the analysis of pharmaceuticals and this could be achieved by utilizing a combinatorial stationary phase prepared by mixing three different molecular imprinting chrial stationary phases equally[9]. Here we get the combinatorial stationary phase by simply connecting columns packed with P1 and P2. **Figure 1** shows the simultaneous chiral separations of Cbz-DL-Ala and Cbz-DL-Ser on the connecting column P1+P2. Non-equimolar quantities of analytes were used for differences of the relative absorbance

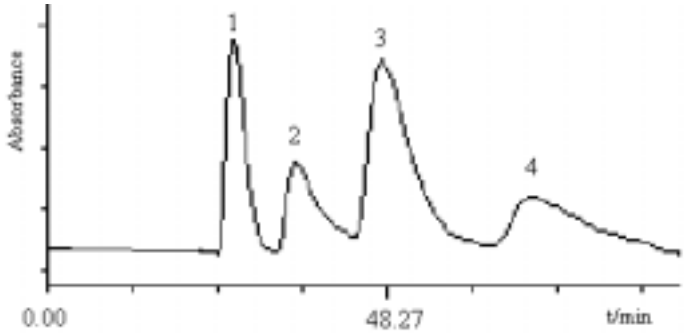
of the analytes. The capacity factors of all the compounds on the column P1+P2 were a little larger than those smaller ones obtained on column P1 or P2. The enantio-selectivity (α) and resolution (f/g) of the enantiomer were obviously increased compared with those on column P1 and P2. Even baseline separation of Cbz-DL-Ala was achieved.

Table 1 Chiral separation of Cbz-DL- Ala and Cbz-DL-Ser on P1, P2 and P1+P2

column	Cbz-DL-Ala				Cbz-DL-Ser			
	k_D'	k_L'	α	f/g	k_D'	k_L'	α	f/g
P1	1.27	1.89	1.49	0.93	2.65	3.40	1.28	0.50
P2	1.76	2.28	1.30	0.55	3.56	5.08	1.43	0.75
P1+P2	1.57	2.43	1.55	1.00	3.63	5.72	1.58	0.89

HPLC conditions: see **Figure 1**

Figure 1 Simultaneous separation of Cbz-DL-Ala and Cbz-DL-Ser on P1+P2



Mobile phase: acetonitrile / acetic acid, (99.5/0.5, v/v); analyte: 20μg Cbz-DL-Ala and 100μg Cbz-DL-Ser in 10μl acetonitrile, detection: 254nm, flow-rate: 0.5mL/min. 1. Cbz-D-Ala, 2. Cbz-L-Ala, 3.Cbz-D-Ser, 4. Cbz-L-Ser;

Potential utility of molecular imprinting polymer was shown in this communication. Combinatorial stationary phase was obtained by simply connecting two columns packed with different imprinted polymer chiral stationary phases. We believe that this approach is superior to the mixing one because the stationary phases are reusable and it is simple by connecting the columns to get the combinatorial phase.

Acknowledgment

This work was supported by the National Natural Science Foundation of China.

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Received 3 June 1999