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Uridine, Thymidine and Inosine Used as Chiral Stationary Phases in HPLC

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In this paper, we present the first enantioseparations research using thymidine, uridine and inosine as chiral stationary phase bonded to silica gel *via* 3-(triethoxysilyl)propyl isocyanate in HPLC. Thymidine and uridine chiral stationary phases possess enantioseparation selectivity for alcohols, amines, ketones and carboxylic acids to some degree in normal-phase and reversed-phase mode. This work indicates that nucleoside or deoxynucleoside can be useful for the separation of enantiomers in the liquid phase as a new kind of chiral stationary phase.

Keywords: Thymidine, Uridine, Inosine, Chiral stationary phase, HPLC.

INTRODUCTION

In nature, chiral phenomenon is ubiquitous. Enantioseparation play an important role in many domains, especially in medicament, nearly 30-40 % are chiral compounds[1] and there are much difference amount the biological activity, toxicity and metabolize mechanism of racemates. Chromatographic separation based on chiral stationary phases (CSPs) represents one of the most direct and facile approaches for the determination of enantiomeric purity[2,3]. In order to develop new stationary phases of enantioselective chromatography, a number of investigations have been done[4-12].

Optical resolution of the amino acid by ultrafiltration using recognition sites of DNA has been investigated[13,14]. The DNA or RNA aptamers, selected against an enantiomer, have become very attractive as new target-specific chiral selectors for HPLC and CE[15,16].

Furthermore, some monosaccharides have been reported used as chiral stationary phases in HPLC[3,17]. DNA, RNA and their aptamers are composed of nucleoside and deoxynucleoside which are constituted with base and ribose or deoxyribose, respectively. To the best of our knowledge, there is no example described of enantiomer separations by nucleoside and deoxynucleoside as chiral selector. Bonding uridine, thymidine and inosine to silica gel *via* an arm of 3-(triethoxysilyl)propyl isocyanate as chiral stationary phase in HPLC, two of them possess good enantioseparation selectivity in normal-phase and reverse-phase mode and there is a chiral discriminating complementary. Fig. 1 showed the structures of uridine, thymidine and inosine. This is a report, for the first time, that nucleoside and deoxynucleoside were bonded on the silica gel used as chiral stationary phase in HPLC.

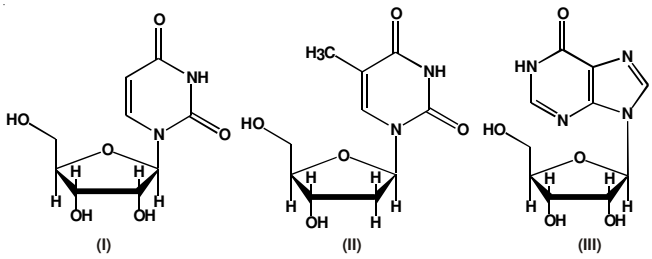

Fig. 1. Structures of uridine(I), thymidine(I.I) and inosine(III)

EXPERIMENTAL

Uridine, thymidine and inosine were purchased from Alfa Aesar. Silica gel (YWG-80, pore size 7 nm, particle size 5 μm) was supplied by Qingdao Ocean Chemical Factory (China). Racemates were obtained from Sigma and Fluka. 3-(Triethoxysilyl)propyl isocyanate was from TCI (Japan). Solvents used in chromatographic experiments were of HPLC grade. All chemicals were at least of analytical grade.

The HPLC system was equipped with a liquid delivery pump and UV-VIS detector (LabTech LC600, USA). A personal computer equipped with a LabTech HPLC Workstation for the LC system was used to process the chromatographic data. Detection was examined at 254 nm.

Synthesis of chiral stationary phases: Uridine, thymidine and inosine chiral stationary phases were prepared as follows:

Equal amount (4 mmol) of 3-(triethoxysilyl)propyl isocyanate was trickled into the flask slowly which stirred with nucleoside or deoxynucleoside in anhydrous pyridine (10 mL) for 0.5 h in ice-bath. The admixture was subjected for stirring at room temperature for 2 h, 3 g of silica gel was added and then the temperature was increased to 80 ºC for 0.5 h. After the reaction was continued for 24 h at 110 ºC, the mixture was filtrated and washed with water, methanol, THF and hexane, respectively. Finally, the product was dried *in vacuo* at 60 ºC for 12 h. Fig. 2 showed the scheme of the synthesis of chiral stationary phases. The IR spectra were used to confirm the formation of bonding between uridine, thymidine and inosine silica gel *via* 3-(triethoxysilyl)propyl isocyanate. The specific C=O vibration of the R-C=ONHR group was obtained at 1700.60, 1699.89 and 1658.84 cm^{-1} for uridine CSP, thymidine CSP and inosine CSP, respectively. The IR spectra also showed the broad and strong vibrational bands in the typical region for vibrations of hydroxyl groups (3444.85 cm^{-1} (uridine CSP); 3420.26 cm^{-1} (thymidine CSP); 3430.54 cm^{-1} (inosine CSP)). The strong vibration bands at 1110.39 and 808.00 cm^{-1} (uridine CSP), 1104.14 and 802.91 cm^{-1} (thymidine CSP) and 1115.56 and 804.66 cm^{-1} (inosine CSP) are detected. They can be assigned to the C-O vibration of the tetrahydrofuran ring groups.

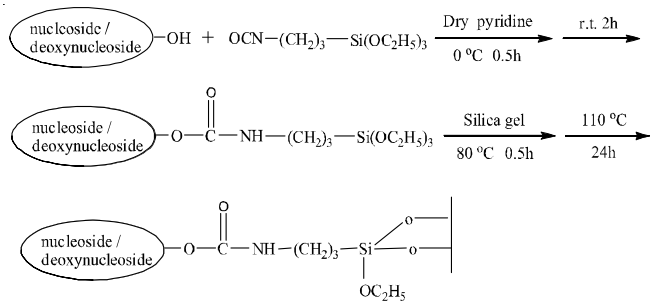

Fig. 2. Scheme of the synthesis of chiral stationary phases

Preparation of HPLC columns: The prepared chiral stationary phases were packed into the stainless steel empty columns (250 mm × 2.0 mm i.d.) respectively by a conventional high-pressure slurry packing procedure with hexane/isopropanol (9:1, v/v) as the slurry solvent using a 1/3 HP liquid pump. The columns were rinsed with ethanol and then equilibrated with hexane/isopropanol (9:1, v/v) as the eluent at a flow rate of 0.1 mL/min before the baseline stabilized.

RESULTS AND DISCUSSION

We investigated the chiral recognition ability of three chira1 columns using 12 racemates, which are 2-phenyl-1-propanol (1), mandelic acid (2), 1-(1-naphthyl)ethanol (3), 1,1'-bi-2-naphthol (4), pindolol (5), 1-(*p*-chlorophenyl)ethanol (6), furoin (7), bendroflumethiazide (8), benzoin (9), praziquantel (10), DNB-(R,S)-leucine (11), propranolol hydrochloride (12) (Fig. 3). Table-1 summarized their retention factors (k_1') for the first eluted enantiomer and separation factors (α). The retention factor k_1' is $(t_1-t_0)/t_0$ and separation factor α is k_2'/k_1' for enantiomer 1-12, where t is the retention time for the analyte and t_0 is the column void time which was determined by 1,3,5-tri-*tert*-butyl-benzene. In an attempt to optimize the separation, the triethylamine or trifluoro acetic acid was added to eluent hexane/isopropanol (90:10, v/v) for some chiral amines or acids and the reverse phase system methanol/water (50:50, v/v) also was tested (Table-1). The relative standard deviation (RSD) of uridine, thymidine and inosine columns for retention factor for five replicate separations of bendroflumethiazide are 0.6, 0.4 and 0.7 %, respectively.

However, in spite of those chiral columns could be used in the reversed-phase mode, the normal-phase resolution was generally better. As can be seen from the Table-l, among the chosen 12 racemic compounds, six enantiomers including alcohols, amines and carboxylic acids *etc*. were separated for uridine colunm, three enantiomers including alcohol, ketone and amine for thymidine and no enantiomers for inosine column since the peak value for five enantiomers were too low to detect and the others only detected single peak. That is to say, uridine column possesses better separation ability than thymidine column towards 12 chiral compounds, but there is a chiral discriminating complementary between them. Unfortunately, inosine column showed little enantioseparation ability.

Fig. 4 exhibited the enantioseparation chromatograms of 2-phenyl-1-propanol on uridine (A), pindolol on thymidine (B) and furoin on thymidine (C) chiral columns, respeetively.

TABLE-1
RETENTION FACTORS (k_1') AND SEPARATION FACTORS (α) FOR ENANTIOMERS ON THREE CHIRAL COLUMNS

Enantiomers	Uridine		Thymidine		Inosine	
	k_1'	α	k_1'	α	k_1'	α
1	1.91	1.32	–	–	1.22	1.00
2	0.98	1.83	–	–	1.20	1.00
3	2.98	1.00	0.81	1.00		
4	9.20	1.00	3.54	1.00	0.30	1.00
5	1.05	1.00	0.75	1.65		
6	–	–	0.65	1.00	1.58	1.00
7	1.29[c]	1.31[c]	3.30[b]	1.49[b]	1.09	1.00
8	0.99[a]	2.07[a]	–	–	0.64	1.00
9	1.88	1.00	1.90	1.87	1.25	1.00
10	11.53	1.00	–	–	–	–
11	0.95[b]	1.83[b]	–	–	–	–
12	1.10	2.25	–	–	–	–

HPLC condition: silica gel, 5 μm; column size: 250 mm × 2.0 mm; eluent: hexane/isopropanol = 90:10; flow rate: 0.1 mL/min; temperature: 30 ºC; detection, 254 nm. a: eluent: hexane/isopropanol/trifluoro acetic acid = 90:10:0.2; b: eluent: hexane/ isopropanol/triethylamine = 90:10:0.2; c: eluent: methanol/water = l:l.

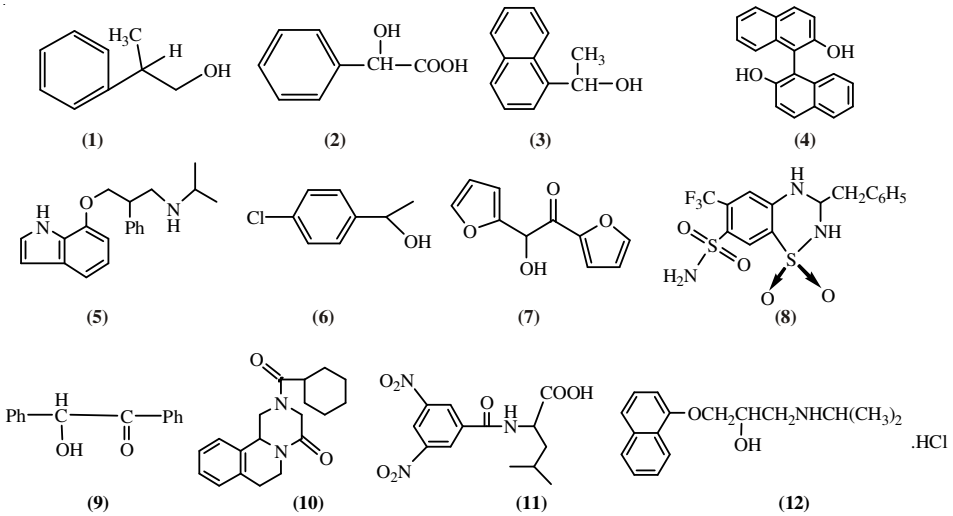

Fig. 3. Molecular structures of chiral compounds

All results indicated the potential of using nucleoside and deoxynucleoside as chiral stationary phases for their enantioselectivity is high enough to reach enantiomer separation. However, the resolution was too low to reach peak separation under the chosen conditions.

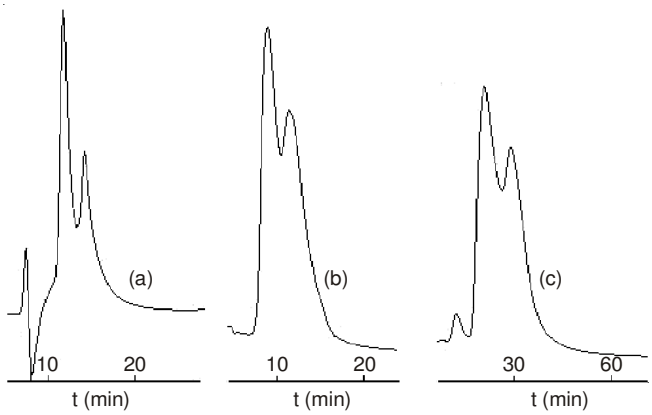

Fig. 4. Enantioseparation chromatograms of racemates. (a): 2-phenyl-1-propanol on uridine column; (b): pindolol on thymidine column; (c): furoin on thymidine column

The chiral recognition mechanism of chiral stationary phases is that besides the dispersion,dipole-dipole and hydrogen-bond forces[18], the steric fit between the chiral saccharide and conformation of the solute molecule may be main interact. This interact would be affected by the kind of monosaccharides and base, the linkage type, the linkage position, *etc.*[14-16,19]. However, it is difficult for the mechanism of chiral recognition to be understood completely. The influence of the chiral micro-environment on the chiral properties of chromatographic systems is far from being understood.

Conclusion

From above comprehensive studies, the optical active nucleoside and deoxynucleoside bonded to silica gel can be used as chiral stationary phases for HPLC, which may be used in normal-phase and reversed-phase mode. They possessed enantioseparation selectivity for alcohols, amines, ketones and carboxylic acids to some extent. The results presented that in the future these valuable chiral materials should be useful for the separation of enantiomers in the liquid phase. For further optimization of the separation efficiency, the derivatization of effective group for the chiral compounds may be adapted and/or a gradient elution is necessary.

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