Enantioseparation of some Antifungals Using Covalently Immobilized Polysaccharide based chiral selectors and polar Organic mobile phases by HPLC

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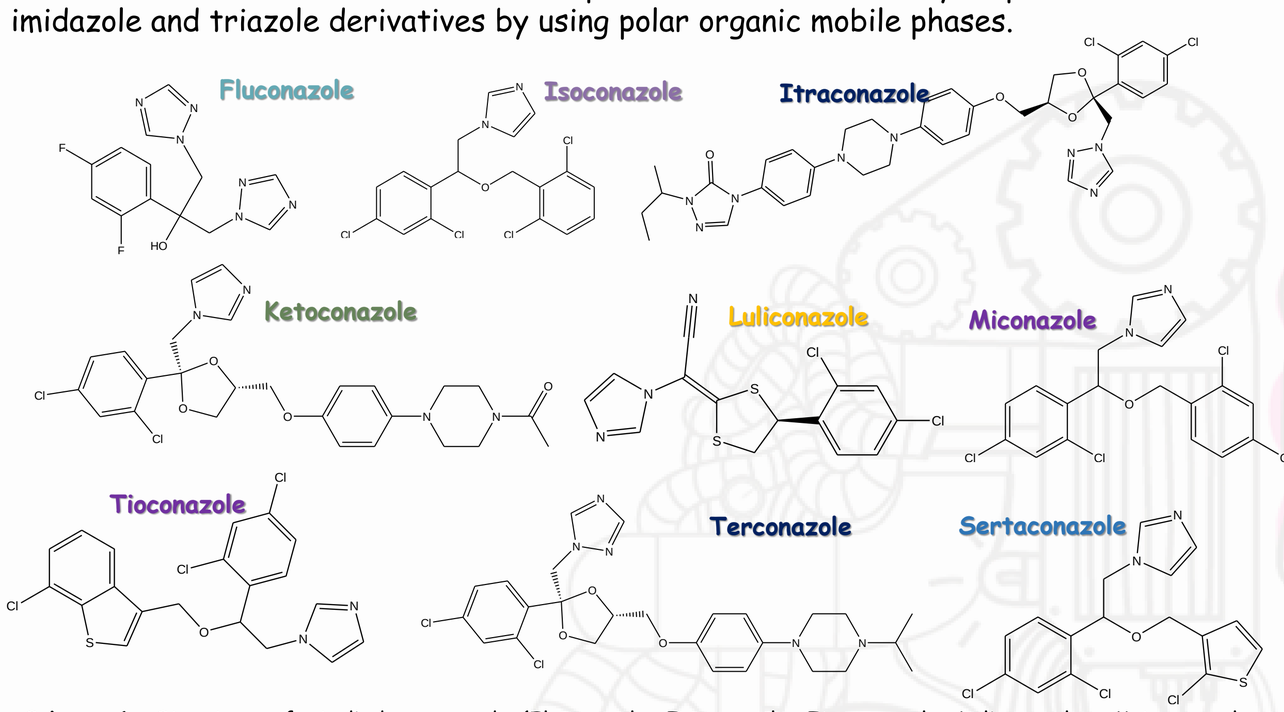
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INTRODUCTION

The separation of enantiomers of chiral imidazole and triazole derivatives in HPLC has been reported by using several different kinds of chiral stationary phases (CSP). However, polysaccharide-based chiral columns have been used in majority of studies.

This study demonstrated the chiral columns based on cellulose 3,5-dichlorophenylcarbamate and amylose 3,5-dimethylphenylcarbamate covalently immobilized to the surface of silica particles can effectively separate the chiral imidazole and triazole derivatives by using polar organic mobile phases.



Scheme 1. Structure of studied compounds (Fluconazole, Isoconazole, Itraconazole, Luliconazole, Ketoconazole, Miconazole, Sertaconazole, Tioconazole , Terconazole, Sertaconazole)

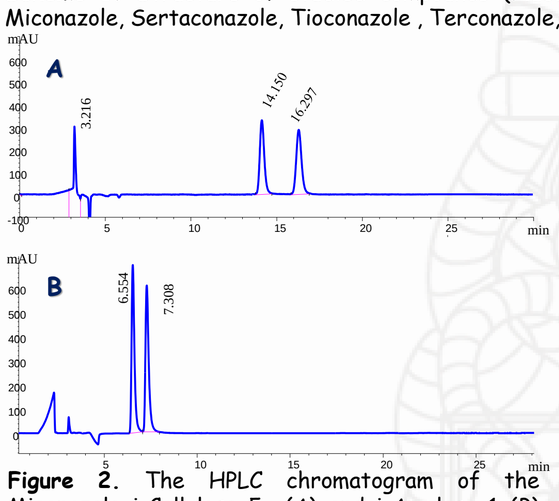


Figure 2. The HPLC chromatogram of the Miconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using ACN as mobile phase

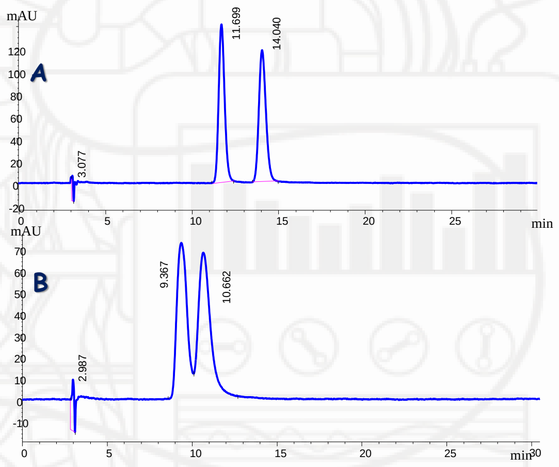


Figure 3. The HPLC chromatogram of the Terconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using EtOH as mobile phase

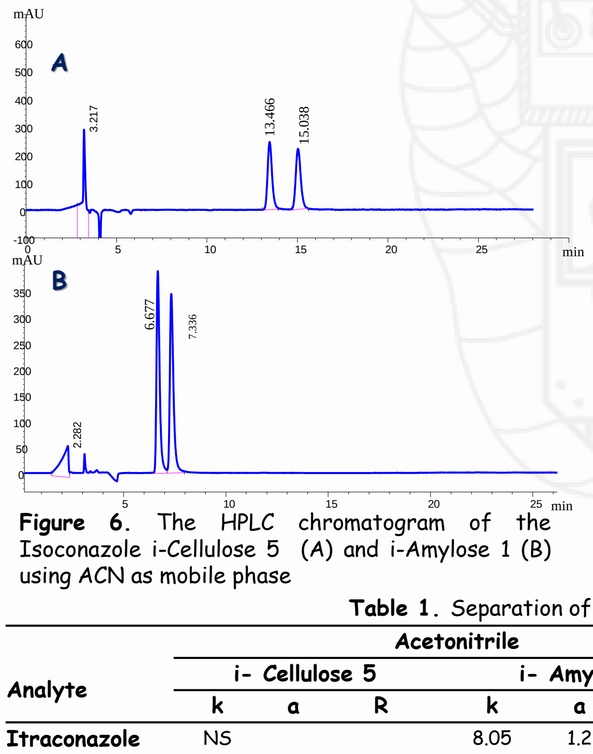


Figure 6. The HPLC chromatogram of the Isoconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using ACN as mobile phase

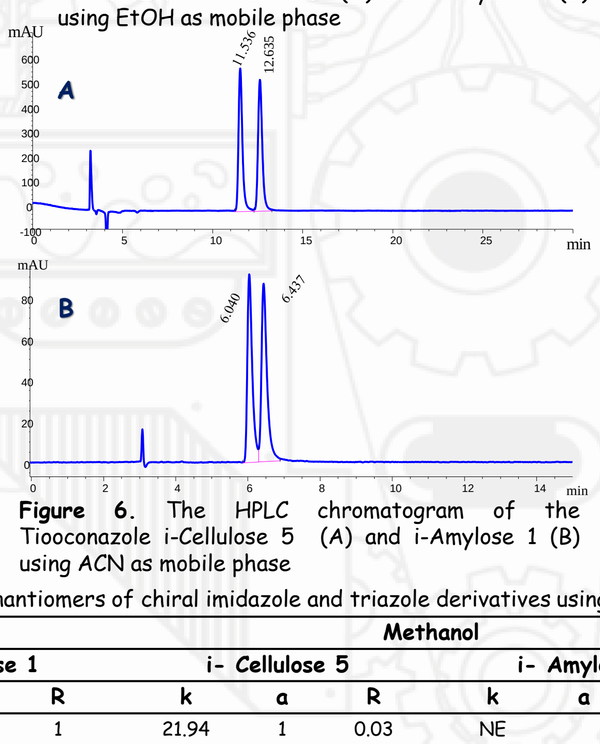


Figure 6. The HPLC chromatogram of the Tiooconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using ACN as mobile phase

MATERIAL & METHOD

Racemic antifungals derivatives used in this study which are illustrated in Figure 1.

HPLC grade solvents such as methanol, acetonitrile, isopropanol and ethanol, were acquired Sigma Aldrich.

Commercially available chiral columns of Lux series from Phenomenex Inc. (Torrance, CA, USA) such as Lux i-Cellulose-5, Lux i- Amylose-1 (Figure 2) with the dimension 4.6x250 mm and 5 μm packing materials were used. The Agilent Technologies HP 1100 series (Santa-Clara, CA, USA) LC system was equipped with a G1379A degasser, G1311A quaternary pump, 61313A auto sampler and G1315B diode array detector. The chromatograms were recorded and the peaks were quantified using an automatic integrator. HPLC separations were performed at 25 °C with 1 mL/min mobile phase flow rate, while detection was performed at 230 nm.

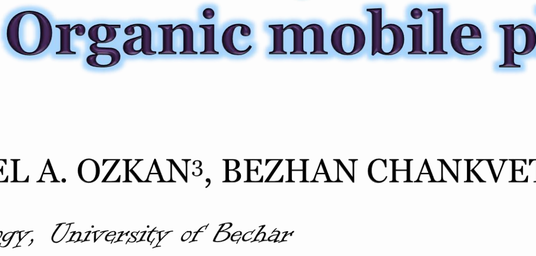


Figure 1. Used chiral stationary phases (CSP)



RESULTS

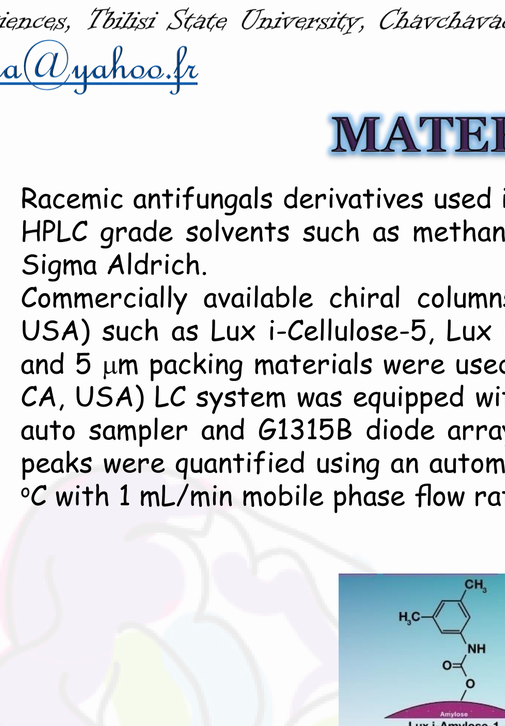


Figure 4. The HPLC chromatogram of the Itraconazole i-Cellulose 5 using ACN (A) and EtOH (B) as mobile phase; i-Amylose 1 using ACN as mobile phase (C)

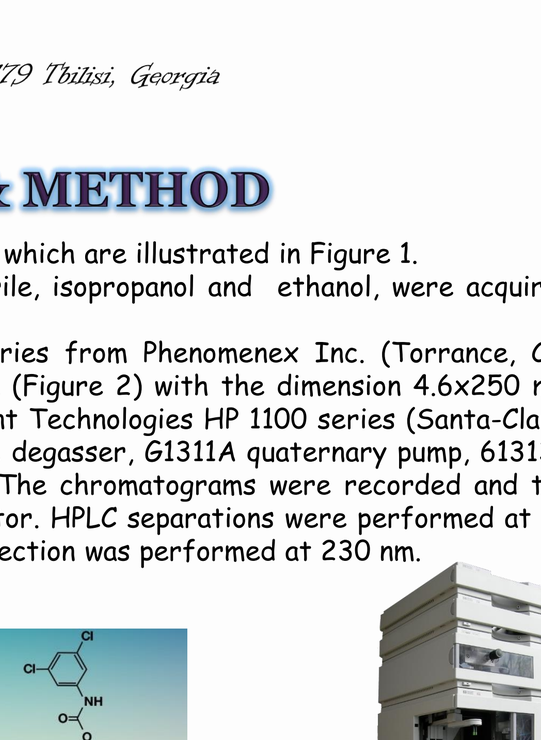


Figure 5. The HPLC chromatogram of the Ketoconazole i-Amylose 1 using ACN (A) (No separation with ACN using i-A1) and ; i-Cellulose 5 using MeOH (B), EtOH (C) as mobile phase

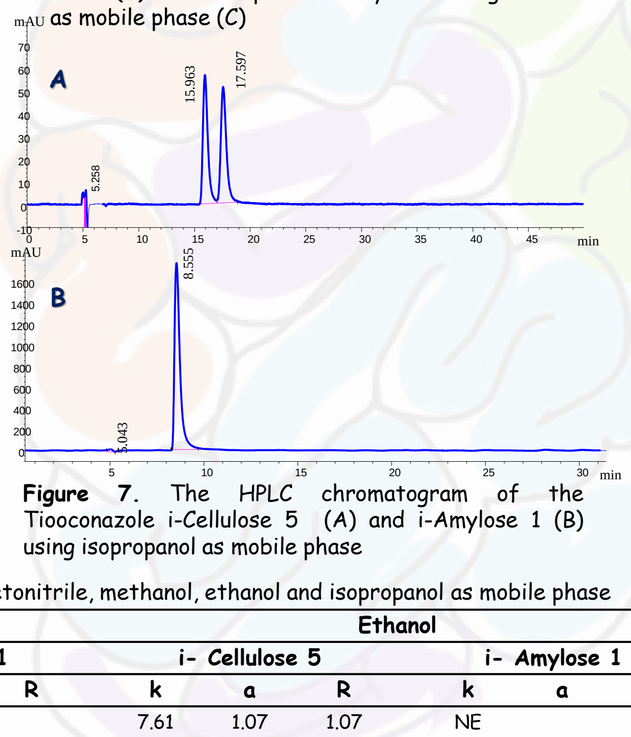


Figure 7. The HPLC chromatogram of the Tiooconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using isopropanol as mobile phase

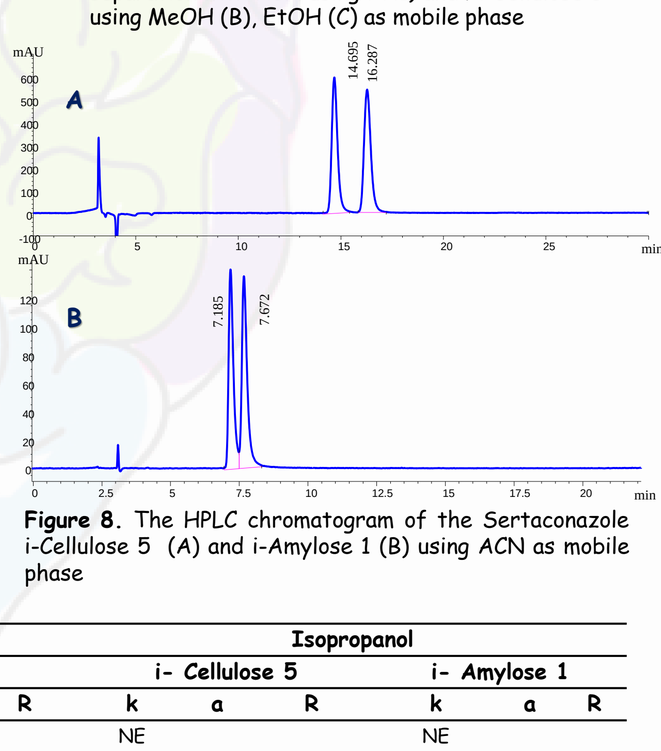


Figure 8. The HPLC chromatogram of the Sertaconazole i-Cellulose 5 (A) and i-Amylose 1 (B) using ACN as mobile phase

Table 1. Separation of enantiomers of chiral imidazole and triazole derivatives using acetonitrile, methanol, ethanol and isopropanol as mobile phase

Analyte	Acetonitrile						Methanol						Ethanol						Isopropanol					
	i- Cellulose 5			i- Amylose 1			i- Cellulose 5			i- Amylose 1			i- Cellulose 5			i- Amylose 1			i- Cellulose 5			i- Amylose 1		
	k	a	R	k	a	R	k	a	R	k	a	R	k	a	R	k	a	R	k	a	R	k	a	R
Itraconazole	NS			8.05	1.2	1	21.94	1	0.03	NE			7.61	1.07	1.07	NE			NE			NE		
				9.9			21.97						8.19	1.02	0.29									
													8.34	1.08	1.26									
													9.05											
Ketoconazole	6.45	1.13	1.05	6.8	1.1	2.15	6.8	1.1	2.15	NS			5.03	1.1	1.73	NS			NE			NS		
	7.42			7.56			7.56						5.62											
Tioconazole	2.6	1.1	2.77	0.94	1.07	1.53	1.62	1.02	0.6	NE			0.6	1.1	0.9	NE			2.04	1.1	1.94	NE		
	2.94			1.04			1.64						0.65						2.35					
Miconazole	3.4	1.13	4.15	2.27	1.12	2.48	1.37	1.03	0.52	1.02	1.02	0.37	0.88	1.06	1.26	NS			2.95	1.14	2.41	NS		
	4.07			2.65			1.45			1.05			0.99						3.52					
Isoconazole	3.27	1.12	3.29	1.92	1.1	2.18	NE			NE			0.79	1.04	1.01	NE			4.5	1.08	1.47	NE		
	3.67			2.21									0.87						2.78					
Sertaconazole	3.88	1.11	2.88	1.39	1.07	1.46	1.28	1.04	0.57	1.11	1.28	6.96	0.77	1.05	1.02	NS			2.83	1.13	2.26	NS		
	4.8			1.55			1.38			1.43			0.99						3.35					
Terconazole	4.59	1.25	5.72	1.71	1.18	1.8	3.8	1.09	0.84	NS			2.8	1.2	3.4	2.14	1.14	1.06	NS			4.47	1.62	1.36
	6.0			3.29			4.23						3.57			2.57						7.87		



CONCLUSION

- The present study illustrates that the polar organic mobile phases offer certain advantages for separation of enantiomers such as short analysis time, high plate numbers and favorable signal to noise ratio.
- ACN found most succesfull mobile phase for the separation of selected antifungals for both immobilized columns.
- Since covalently immobilized chiral columns offer good stability in pure polar organic solvents, separation of enantiomers of antifungals under this study was first evaluated in polar organic solvents for related compounds.

References

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