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Comparative study of the enantiomeric resolution of chiral antifungal drugs econazole, miconazole and sulconazole by HPLC on various cellulose chiral columns in normal phase mode

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

The chiral resolution of (±)-econazole, (±)-miconazole and (±)-sulconazole on the columns containing different cellulose derivatives namely Chiralcel OD, OJ, OB, OK, OC and OF in normal phase mode has been described. The mobile phase used was hexane–2-propanol–diethylamine (425:74:1, v/v/v). The flow rates of the mobile phase used were 0.50, 1.00 and 1.50 ml/min. The values of the separation factor (α) of the resolved enantiomers of econazole, miconazole and sulconazole on chiral phases were ranged from 1.07 to 2.50 while the values of resolution factor (R_s) varied from 0.17 to 3.90. The chiral recognition mechanisms between the analytes and the chiral selectors are discussed.

Keywords: Chiral resolution; Antifungal agents; Econazole; Miconazole; Sulconazole; Cellulose chiral stationary phases

1. Introduction

The different pharmacological activities of the enantiomers has created an interest to study the pharmacological and toxicological properties of the enantiomers e.g. drugs, pharmaceuticals, agrochemicals etc. [1–3]. Only about 20–25% of the chiral pharmaceuticals are sold as pure enantiomers. The US Food and Drug Administration has issued the order to pharmaceutical and agrochemical industries to specify the enantiomeric purity of the chiral compounds [4]. In view of this, the enantiomeric resolution of the chiral compounds became an urgent need of pharmaceutical, agrochemical and other chemical based industries. Therefore, there is an increasing demand for the direct methods of chiral resolution of enantiomers of the chiral compounds.

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The chromatographic resolutions of enantiomers, particularly by high performance liquid chromatography (HPLC), have gained a great reputation in the last 15 years and have become a practically useful method for determining optical purity and for obtaining enantiomers [5–8]. Various chiral columns have been used for the enantiomeric resolution of a wide variety of racemates [8]. Among these the chiral columns containing cellulose derivatives have a wide range of applications [7–11]. Among the various imidazole antifungal agents econazole, miconazole and sulconazole (Fig. 1) are supposed to be the most commonly used due to their therapeutic properties [12]. The enantiomeric resolution of these antifungal agents have been reported by HPLC [13–15] on cyclodextrins and by capillary electrophoresis on *N*-dodecoxycarbonylvaline columns [16,17]. In this study the attempt was made to resolve the enantiomers of econazole, miconazole and sulconazole antifungal agents on various commercially available cellulose based chiral columns by HPLC.

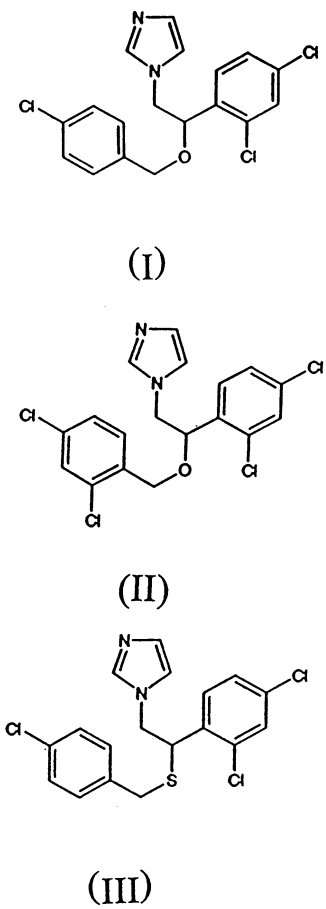

Fig. 1. The chemical formulae of antifungal agents econazole (**I**), miconazole (**II**), and sulconazole (**III**).

2. Experimental

2.1. Chemicals and reagents

The racemic mixtures of econazole, miconazole and sulconazole were obtained from Sigma Chem. Co., USA. The solutions of the individual antifungal agents (1 mg/ml) were prepared in the mobile phase. Hexane and 2-propanol of HPLC grade were purchased from Fisher Scientific (Fairlawn, NJ, USA). Diethylamine was also purchased from Sigma Chem. Co., USA.

2.2. Chromatographic conditions

About 20 μl of each of the solutions were injected on to a HPLC system consisting of Waters solvent delivery pump (model 510, Milford, MA, USA), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484) and Waters integrator (model 740). The order of elution of the enantiomers was determined by using a polarimetric detector (Shodex OR-1, J.M. Sciences Inc., Buffalo, USA). The columns used were: Chiralcel OB (Cellulose tris benzoate), Chiralcel OJ (Cellulose tris 4-methylbenzoate), Chiralcel OK (Cellulose tris cinnamate), Chiralcel OC (Cellulose tris phenylcarbamate), Chiralcel OD (Cellulose tris 3,5-dimethylphenylcarbamate) and Chiralcel OF (Cellulose tris 4-chlorophenylcarbamate). All the columns were 25×0.46 cm size packed with a chiral stationary phase of 10 μm size and were obtained from Daicel Chemical Industries, Tokyo, Japan. The mobile phase used in this study was hexane–2-propanol–diethylamine (425:74:1, v/v/v). The mobile phase was filtered and degassed before use. The flow rates of the mobile phase were 0.50, 1.0 and 1.50 ml/min

Table 1
The chromatographic parameters, k, α, and R_s for enantiomeric resolution of econazole, miconazole and sulconazole on Cellulose chiral stationary phases using hexane–2-propanol–diethyl amine (425:74:1, v/v/v) as the mobile phase with 0.50 ml/min flow rate

	k_1 (−)	k_2 (+)	α	R_s
Chiralcel OF				
Econazole	8.55	10.95	1.28	3.37
Miconazole	7.51	9.09	1.21	3.30
Sulconazole	12.87	15.86	1.23	3.90
Chiralcel OC				
Econazole	6.39	7.35	1.15	1.90
Miconazole	5.31	6.03	1.14	1.51
Sulconazole	nr			
Chiralcel OD				
Econazole	nr			
Miconazole	6.76	7.26	1.07	0.80
Sulconazole	15.33	20.66	1.35	3.61
Chiralcel OJ				
Econazole	nr			
Miconazole	11.44	15.00	1.31	0.33
Sulconazole	21.26	33.84	1.59	2.39
Chiralcel OB				
Econazole	2.36	3.58	1.52	0.30
Miconazole	1.43	3.31	2.32	0.21
Sulconazole	1.49	3.72	2.50	0.39
Chiralcel OK				
Econazole	nr			
Miconazole	4.08	5.20	1.28	0.90
Sulconazole	nr			

nr, not resolved. For details see Section 2.

separately. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at 23 ± 1 °C. The detection was carried out at 250 nm. The chromatographic parameters such as capacity factor (k), separation factor (α) and resolution factor (R_s) were calculated.

3. Results and discussion

The chromatographic parameters, k, α, and R_s for the resolved enantiomers of (±)-econazole, (±)-miconazole and (±)-sulconazole at 0.50, 1.00 and 1.50 ml/min flow rate are given in Tables 1–3, respectively. The typical chromatograms of the resolved enantiomers of these antifungal agents on Chiralcel OF column is shown in Fig. 2. It is clear from Tables 1–3 that the econazole, miconazole and sulconazole have been resolved successfully on different cellulose columns. The order of elution was confirmed by using the polarimetric detector. It has been observed that the (−)-enantiomer eluted first followed by (+)-enantiomer for the three antifungal agents studied. A variation in the chromatographic parameters was carried out to obtain the best resolution. To optimize the chromatographic conditions, mixtures of various alcohols, acetonitrile, hexane, diethylamine etc. were tried but no good resolution could be achieved. As a result of exten-

Table 2
The chromatographic parameters, k, α, and R_s for enantiomeric resolution of econazole, miconazole and sulconazole on Cellulose chiral stationary phases using hexane–2-propanol–diethyl amine (425:74:1, v/v/v) as the mobile phase with 1.0 ml/min flow rate

	k_1 (−)	k_2 (+)	α	R_s
Chiralcel OF				
Econazole	8.56	11.00	1.29	3.78
Miconazole	7.51	9.07	1.21	3.23
Sulconazole	12.87	15.90	1.24	3.80
Chiralcel OC				
Econazole	6.34	7.34	1.16	1.60
Miconazole	5.32	6.09	1.15	1.23
Sulconazole	nr			
Chiralcel OD				
Econazole	nr			
Miconazole	6.79	7.30	1.08	0.59
Sulconazole	15.49	20.72	1.34	3.41
Chiralcel OJ				
Econazole	nr			
Miconazole	11.64	15.23	1.31	0.21
Sulconazole	21.66	34.45	1.59	2.22
Chiralcel OB				
Econazole	2.36	3.59	1.52	0.21
Miconazole	1.43	3.34	2.34	0.17
Sulconazole	1.45	3.66	2.52	0.25
Chiralcel OK				
Econazole	nr			
Miconazole	4.31	5.24	1.22	0.80
Sulconazole	nr			

nr, Not resolved. For details see Section 2.

Table 3
The chromatographic parameters, k, α, and R_s for enantiomeric resolution of econazole, miconazole and sulconazole on Cellulose chiral stationary phases using hexane–2-propanol–diethyl amine (425:74:1, v/v/v) as the mobile phase with 1.50 ml/min flow rate

	$k_1(-)$	$k_2(+)$	α	R_s
Chiralcel OF				
Econazole	8.84	11.31	1.28	3.00
Miconazole	10.69	12.76	1.19	2.60
Sulconazole	13.30	16.40	1.23	3.10
Chiralcel OC				
Econazole	6.41	7.38	1.15	1.60
Miconazole	5.26	6.08	1.16	1.10
Sulconazole	nr			
Chiralcel OD				
Econazole	nr			
Miconazole	nr			
Sulconazole	21.39	28.61	1.34	3.26
Chiralcel OJ				
Econazole	nr			
Miconazole	nr			
Sulconazole	21.65	34.43	1.59	2.00
Chiralcel OB				
Econazole	nr			
Miconazole	nr			
Sulconazole	1.44	3.80	2.64	0.20
Chiralcel OK				
Econazole	nr			
Miconazole	nr			
Sulconazole	nr			

nr, Not resolved. For details see Section 2.

sive experimentation the chromatographic conditions were optimized.

The resolution of these antifungal agents was in the order of OF > OC > OD > OJ > OB > OK. This behavior may be explained on the basis of the magnitude of the different hydrogen bondings since the hydrogen bonding, between the antifungal agents and cellulose derivatives, apparently increases in the same order. More hydrogen bondings are formed between the analytes and the cellulose carbamate derivatives in Chiralcel OD, OC and OF, in comparison to the ones formed between the analytes and the cellulose ester derivatives. It is of interest to mention that the cellulose carbamate derivatives were more efficient in the enantiomeric resolution of the racemic antifungal drugs used in this study than the cellulose ester derivatives. Sulconazole is more retained than econazole and miconazole. It may be due to the coordination bonding between sulconazole and the chiral selectors due the presence of d vacancy in the sulphur atom of sulconazole. Miconazole is less retained than econazole and it may be due to the steric effect of the two 2,4-dichlorophenyl groups in miconazole. Attempts have been made to find out the effect of flow rates on the enantiomeric resolution of these antifungal drugs. The enantiomeric resolution has been carried out using 0.50, 1.00 and 1.50 ml/min flow rates and Tables 1–3 show that the best resolution occurred using 1.00 ml/min flow rate. It has also been observed that the complete resolution of econazole and miconazole occurred on Chiralcel OF and OC columns while the complete resolution of sulconazole has been achieved on Chiralcel OF, OD and OJ columns. Partial resolution of these antifungal agents was also observed on Chiralcel OB and OK columns using 0.50, 1.00 and 1.50 ml/min flow rates (Tables 1–3). The values of the capacity factors are higher on Chiralcel OJ than on Chiralcel OB column. It could be due to the presence of the methyl group on phenyl ring in Chiralcel OJ column in comparison to Chiralcel OB which increases the electron density on the phenyl ring by inductive effect. The higher electron density in Chiralcel OJ column provides stronger π–π bonding in comparison to the π–π bonding in Chiralcel OB column which resulted into the higher capacity factors of antifungal agents on Chiralcel OJ column. Generally, the retention times of econazole, miconazole and sulconazole are high in all the chromatographic systems. It may be due the greater strength of the interactions (hydrogen, π–π, dipole–dipole induced and coordination bondings) between antifungal agents and chiral selectors. The greater strength of bonding is due to the presence of six to seven electronegative atoms and three aromatic rings (two phenyl rings and one imidazole ring) in these antifungal agents available for interaction with various functional groups on the cellulose chiral selectors.

The chiral recognition mechanism at a molecular level on the cellulose based CSPs is still unclear although it has been reported that the chiral resolution by these CSPs is achieved through the different hydrogen, π–π and dipole–dipole induced interactions between the chiral stationary phase and the analytes enantiomers [18–20]. The cellulose based chiral stationary phases are semi-synthetic polymers which contain the polymeric chains of derivatized D-(+) glucose residues in β-1,4 linkage and these chains lie side by side in a linear fashion. The structure of the antifungal agents (Fig. 1) contains electronegative atoms namely nitrogen, oxygen, sulphur and chlorine along with three aromatic rings (two phenyl rings and one imidazole ring). Therefore, the resolution of the enantiomers of these antifungal agents occurred due to the different hydrogen bonding and dipole–dipole induced interactions of different magnitudes between the electronegative atoms of antifungal agents and cellulose stationary phases. Furthermore, it has also been reported [18,19] that the π–π interactions between the substituted phenyl moieties of cellulose based chiral stationary phases and the aromatic rings of the analytes plays an important role in the chiral resolution mechanisms. Therefore, the three aromatic rings (two phenyl rings and one imidazole ring) of each enantiomers of antifungal agents fit stereogenically in the different fashion into the chiral grooves of the stationary phases which is stabilized by the π–π interactions of different magnitude for both (+) and (−) enantiomers resulting in the resolution of enantiomers. It is also of interest to note that coordination bonding in sulconazole does apparently play a role in the enantiomeric resolution of this drug due to the presence of sulphur atom.

4. Conclusion

This study indicates that the capacities of the enantiomeric resolution of studied antifungal

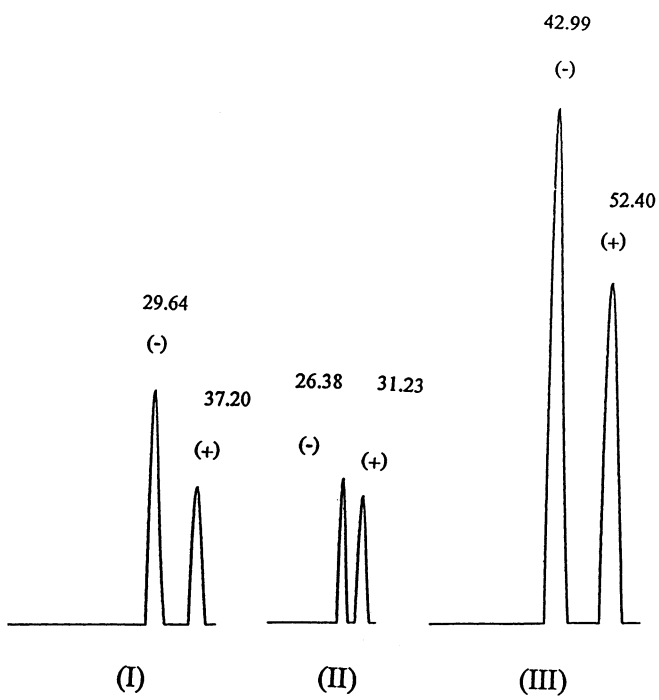

Fig. 2. The chromatograms showing the resolution of enantiomers of econazole (**I**), miconazole (**II**), sulconazole (**III**) on Chiralcel OF column using hexane–2-propanol–diethylamine (425:74:1, v/v/v) as the mobile phase with 1.00 ml/min flow rate.

agents on several cellulose based CSPs were in the order of Chiralcel OF > OC > OD > OJ > OB > OK. Taking into the consideration the results obtained, one can conclude that the enantiomeric resolution of antifungal agents on these chiral stationary phases is governed by hydrogen bonding, π–π and dipole induced dipole interactions. Besides, coordination bonding is apparently responsible for enantiomeric resolution of sulconazole.

The reported HPLC system can be used for the resolution of (±)-econazole, (±)-miconazole and (±)-sulconazole on a semi-preparative scale for further pharmacological investigations of the individual enantiomer of these drugs.

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