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Comparison of the chiral resolution of econazole, miconazole, and sulconazole by HPLC using normal-phase amylose CSPs

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Abstract Resolution of the enantiomers of (±)-econazole, (±)-miconazole, and (±)-sulconazole has been achieved on different normal-phase chiral amylose columns, Chiralpak AD, AS, and AR. The mobile phase used was hexane–2-propanol–diethylamine, 400:99:1 (v/v). The flow rates of the mobile phase used were 0.50 and 1.00 mL min^{-1}. The α values for the resolved enantiomers of econazole, miconazole, and sulconazole on the chiral phases were in the range 1.63 to 1.04; the R_s values varied from 5.68 to 0.32.

Introduction

Imidazole derivatives are used as antifungal agents. Among the various antifungal agents econazole, miconazole, and sulconazole are regarded as the best, because of their high tolerance limit and the rapid mode of action [1–3]. These antifungal agents contain one chiral centre (Fig. 1) and are clinically used as a racemic mixture. It is well known that the enantiomers differ in their pharmacological and toxicological activities – one of the enantiomers can be inactive or toxic [4]. These properties of the enantiomers have created an interest in the study of the pharmacological and toxicological properties of the individual enantiomers of, e.g., pharmaceuticals and agrochemicals, etc. [5, 6]. The US Food and Drug Administration have issued an order to the pharmaceutical and agrochemical industries to specify the enantiomeric purity of optically active compounds before they are marketed [7]. For these reasons the enantiomeric resolution of econazole, miconazole, and sulconazole is of great interest to the pharmaceutical industries for possible racemic switch, i.e. replacing the racemate with one of the enantiomers, to improve the efficacy of the compound [8].

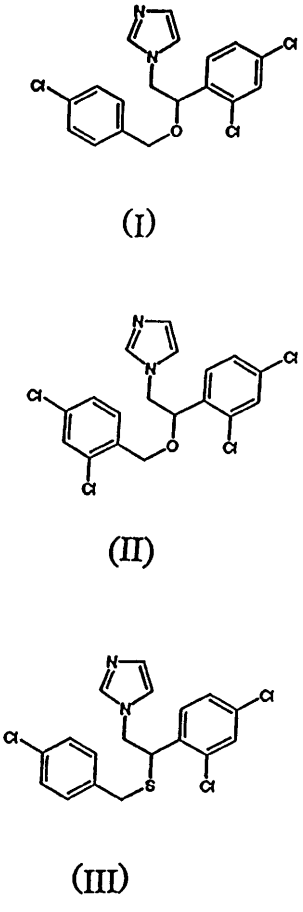

Fig. 1 The chemical formulae of the antifungal agents econazole (I), miconazole (II), and sulconazole (III)

Polysaccharide-based chiral stationary phases, e.g. cellulose and amylose CSPs, have been widely used for resolution of the enantiomers of a large variety of racemates by liquid chromatography [9–14]. It has also been reported that the amylose CSP is a better chiral selector than cellulose, because of its more helical structure [15]. Resolution of the enantiomers of econazole, miconazole, and

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sulconazole on amylose columns was, therefore, investigated.

Experimental

Chemicals and reagents. Racemic mixtures of econazole, miconazole, and sulconazole were obtained from Sigma, USA. Solutions (1 mg mL^{-1}) of these antifungal agents were prepared in the mobile phase. Hexane and 2-propanol of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Diethylamine was also purchased from Sigma.

Chromatographic conditions. The solutions (20 μL of each) were injected in to an HPLC system comprising a Waters solvent delivery pump (model 510), a Waters injector (model WISP 710B), a Waters tunable absorbance detector (model 484), and Waters integrator (model 740). The order of elution of the enantiomers was confirmed by use of a polarimetric detector (Shodex OR-1; J.M. Sciences, Buffalo, USA). The columns used, Chiralpak AD (25 cm × 0.46 cm, particle size 10 μm) [Amylose tris 3,5-dimethylphenylcarbamate], Chiralpak AR (25 cm × 0.46 cm, particle size 10 μm) [Amylose tris *R*-α-methylphenylcarbamate], and Chiralpak AS (25 cm × 0.46 cm, particle size 10 μm) [Amylose tris *S*-α-methylphenylcarbamate], were obtained from Daicel Chemical Industries, Tokyo, Japan. The structures of these chiral stationary phases are shown in Fig. 2. The mobile phase was hexane–2-propanol–diethylamine, 400:99:1 (v/v); the mobile phase was filtered and degassed before the use. The flow rates of the mobile phase were 0.50 and 1.0 mL min^{-1}. The chart speed was kept constant at 0.1 cm min^{-1}. All the experiments were performed at 23 ± 1 °C. The detection was performed at 250 nm. The chromatographic data capacity factor (k), separation factor (α), and resolution factor (R_s) were calculated.

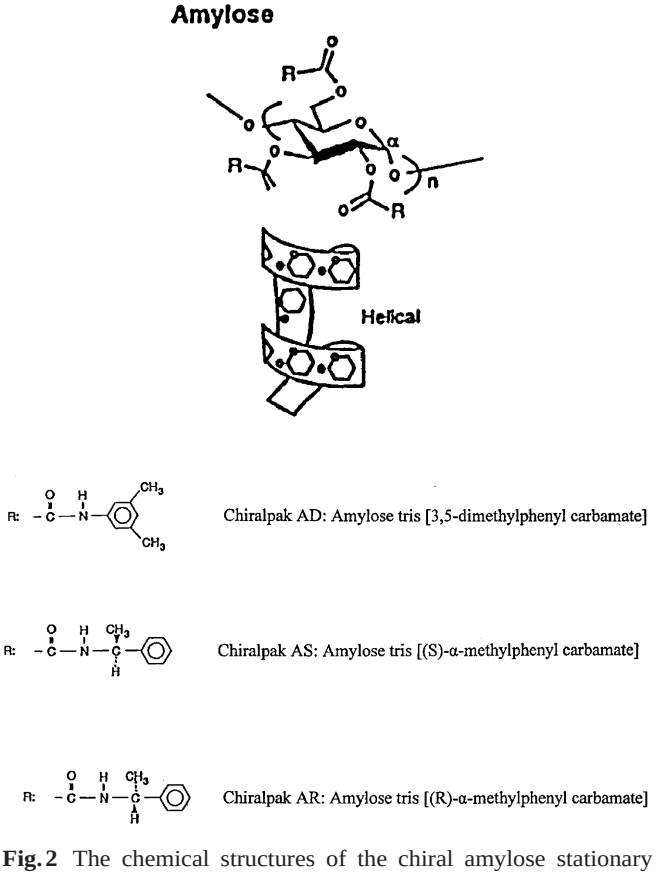

Fig. 2 The chemical structures of the chiral amylose stationary phases

Results and discussion

The chromatographic data capacity factor (k), separation factor (α), and resolution factor (R_s) for the resolved enantiomers of (±)-econazole, (±)-miconazole, and (±)-sulconazole at flow rates of 0.50 and 1.00 mL min^{-1} are given in Tables 1 and 2, respectively. Typical chromatograms of the resolved enantiomers of these antifungal agents on the Chiralpak AS column using flow rates of 0.50 and 1.00 mL min^{-1} are given in Figs. 3 and 4, respectively. It is clear from Tables 1 and 2 that the econazole, miconazole, and sulconazole have been resolved successfully on the different amylose columns. The order of elution was confirmed by use of a polarimeter. It has been observed that the (–) was enantiomer eluted before the (+) enantiomer for all three antifungal agents.

Table 1 Chromatographic data – capacity factor (k), separation factor (α), and resolution factor (R_s) – for resolution of the enantiomers of econazole, miconazole, and sulconazole on amylose chiral stationary phases with hexane–2-propanol–diethylamine, 400:99:0.1 (v/v), as mobile phase at a flow rate of 0.5 mL min^{-1}

	k_1 (–)	k_2 (+)	α	R_s
Chiralpak AS				
Econazole	6.36	10.35	1.63	5.32
Miconazole	5.28	8.21	1.56	4.69
Sulconazole	9.71	14.4	1.48	5.68
Chiralpak AD				
Econazole	6.79	7.15	1.05	1.42
Miconazole	5.25	5.56	1.06	1.26
Sulconazole	8.27	9.62	1.16	3.60
Chiralpak AR				
Econazole	5.91	6.35	1.07	0.45
Miconazole	5.11	5.35	1.05	0.32
Sulconazole	nr			

nr: not resolved

Table 2 Chromatographic data – capacity factor (k), separation factor (α) and resolution factor (R_s) – for resolution of the enantiomers of econazole, miconazole, and sulconazole on amylose chiral stationary phases with hexane–2-propanol–diethylamine, 400:99:0.1 (v/v), as mobile phase at a flow rate of 1.0 mL min^{-1}

	k_1 (–)	k_2 (+)	α	R_s
Chiralpak AS				
Econazole	6.43	10.42	1.62	2.66
Miconazole	5.38	8.27	1.54	2.89
Sulconazole	9.72	14.34	1.48	3.08
Chiralpak AD				
Econazole	6.29	7.29	1.05	0.37
Miconazole	5.25	5.57	1.06	0.32
Sulconazole	9.35	9.71	1.04	1.36
Chiralpak AR				
Econazole	6.11	6.56	1.07	0.40
Miconazole	nr			
Sulconazole	nr			

nr: not resolved

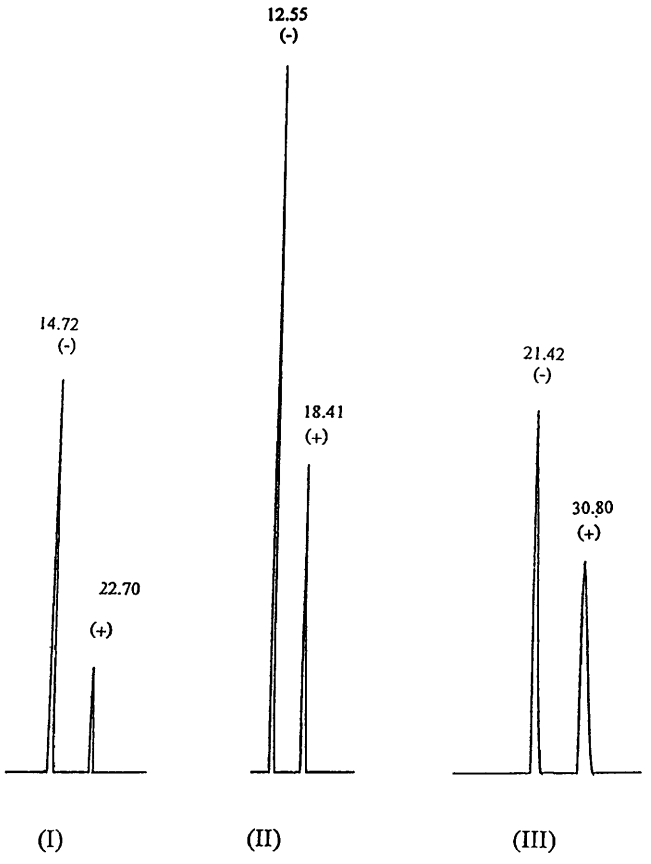

Fig. 3 Chromatograms showing the resolution of the enantiomers of econazole (I), miconazole (II), and sulconazole (III) on the Amylose AS column with hexane–2-propanol–diethylamine, 400:99:0.1 (v/v), as mobile phase at a flow rate of 0.5 mL min^{-1}

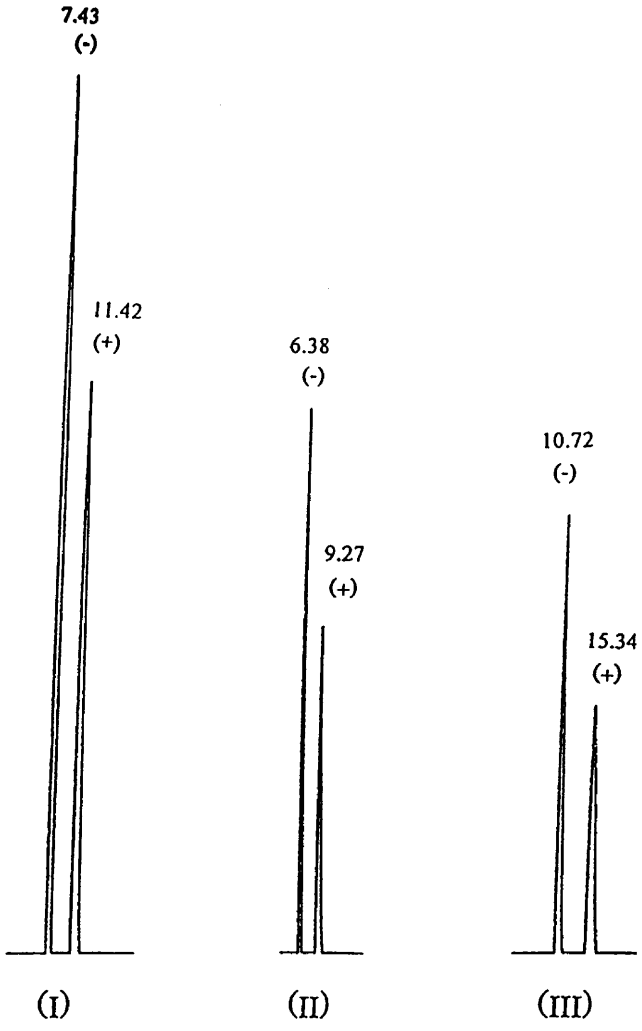

Fig. 4 Chromatograms showing the resolution of the enantiomers of econazole (I), miconazole (II), and sulconazole (III) on the Amylose AS column with hexane–2-propanol–diethylamine, 400:99:0.1 (v/v), as mobile phase at a flow rate of 1.0 mL min^{-1}

The chromatographic conditions were varied to obtain the best resolution. Mixtures of different alcohols, acetonitrile, hexane, and diethylamine, etc. were tested but only poor resolution was achieved. As a result of extensive experiments the optimized chromatographic conditions were developed and reported herein.

The resolution of the antifungal agents was in the order Chiralpak AS > AD > AR. Chiral resolution on these phases can be explained on the basis of interactions and steric effects. Resolution of enantiomers on amylose chiral stationary phases is supposed to be a result of hydrogen-bonding-, π–π and dipole–dipole-induced interactions between the chiral stationary phases and the analytes. Figure 2 indicates that Chiralpak AS and Chiralpak AR contain one extra chiral carbon than Chiralpak AD. The better resolution on Chiralpak AS compared with Chiralpak AD might be because of the presence of this extra chiral carbon in Chiralpak AS which exerts the steric effects. It was also observed that the order of elution of the analytes was not reversed when using Chiralpak AS and AR columns; this indicates that the chirality at the methyl group does not affect the resolution but simply exerts a steric effect.

Resolution of the enantiomers of these antifungal agents is better on Chiralpak AS than on Chiralpak AR. This could be explained by the different steric effects of these two CSPs , because of the different configuration of the chiral carbon. From these findings it can also be concluded that the *S* configuration of Chiralpak AS CSP is better than the *R* configuration for chiral resolution. It is interesting to note that sulconazole is more retained than econazole and miconazole. The reason for this might be strong coordination bonding between sulconazole and the stationary phases, because sulfur has the capacity for stronger coordination bonding than oxygen. Miconazole is less retained than econazole, because of the steric effect in miconazole exerted by the extra chlorine atom in comparison to econazole. Miconazole is, furthermore, less retained than sulconazole because of a steric effect (owing to the presence of one extra chlorine atom in miconazole) and the poor coordination bonding in miconazole.

Enantiomer resolution was performed using flow rates of 0.50 and 1.00 mL min^{-1} and it is clear from Tables 1 and 2 that the best resolution was achieved with a flow

rate of 0.50 mL min^{-1}. Complete resolution of econazole, miconazole, and sulconazole was achieved on Chiralpak AS and AD and partial resolution on Chiralpak AR. The α values of the resolved enantiomers of econazole, miconazole, and sulconazole on amylose chiral phases were in the range 1.63 to 1.04; R_s values varied from 5.68 to 0.32. This clearly indicates that the amylose chiral stationary phases are suitable for resolution of the enantiomers antifungal agents.

Polysaccharides such as cellulose and amylose are the most readily available optically active polymers and are known to have chiral discrimination capability. Native cellulose and amylose are not practically useful CSP, however, because of insufficient optical resolving power. The polysaccharides are, on the other hand, easily converted to a variety of derivatives, e.g. tris-esters and tris-carbamates, by reaction of active hydroxyl groups with appropriate reagents. Several cellulose- and amylose-based chiral stationary phases are available and have been used for resolution of the enantiomers of a wide variety of racemates by liquid chromatography [16–28]. Amylose is a semisynthetic polymer which contains polymeric chains of derivatized D–(+) glucose residues in α-1,4 linkage. These chains lie side by side in a helical fashion. The better resolving capacity of amylose might be because the amylose CSPs are more helical in nature and have well defined grooves, in contrast with the corresponding cellulose analogues which seem to be more linear and more rigid in nature [15], and hence afford a more chiral environment to the antifungal agents.

The mechanism of chiral recognition, on a molecular level, of the cellulose-based CSPs is still unclear although it has been reported that chiral resolution by these CSPs is achieved as a result of different hydrogen-bonding, π-π and dipole–dipole induced interactions between the chiral stationary phase and the enantiomers [29–31]. The structures of the antifungal agents (Fig. 1) contain electronegative atoms – nitrogen, oxygen, sulfur, and chlorine – and three aromatic rings. Resolution of the enantiomers of the antifungal agents was, therefore, achieved because of the different magnitudes of these hydrogen-bonding and dipole–dipole induced interactions between the amylose stationary phases and the electronegative atoms of the different enantiomers of the antifungal agents. It has also been reported [29, 30] that π–π interactions of different magnitude occur between the substituted phenyl moieties of amylose-based chiral stationary phases and the aromatic rings of the analytes. The three aromatic rings of each enantiomer of the antifungal agents fit stereogenically, in a different fashion, into the chiral grooves of the stationary phases and the complex formed is stabilized by π-π interactions of different magnitude for the (+) and (–) enantiomers and, hence, resolution of enantiomers occurs. It is interesting to note that coordination bonding in sulconazole plays an important role in the resolution of the enantiomers of sulconazole, as discussed earlier.

Conclusion

Resolution of the enantiomers of the studied antifungal agents on three amylose-based CSPs was in the order Chiralpak AS > AD > AR. From the results obtained it can be concluded that resolution of the enantiomers of antifungal agents on these chiral stationary phases is governed by hydrogen, π–π and dipole–induced-dipole interactions. The steric effect arising from the chiral carbons in Chiralpak AS and AR and the two methyl groups attached to phenyl ring of Chiralpak AD also plays a crucial role in the resolution of the enantiomers of these antifungal agents on amylose CSPs. Coordination bonding is also responsible for resolution of the enantiomers of sulconazole.

The reported HPLC system is simple, fast, and reproducible and can be used to resolve (±)-econazole, miconazole, and sulconazole on a semi preparative scale for further pharmacological investigations of the individual enantiomers of these drugs.

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