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Enantiomeric resolution of chiral imidazole derivatives using capillary electrophoresis with cyclodextrin-type buffer modifiers

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Abstract

Enantiomeric resolutions of some chiral pharmaceuticals containing the imidazole (1,3-diazole) moiety were carried out using capillary electrophoresis. Various native cyclodextrins (α-, β- and γ-cyclodextrin) and derivatized cyclodextrins (hydroxypropyl-, and sulfobutyl ether-β-cyclodextrin) were used as chiral buffer modifiers. The effects of the cavity size, the structure and the charge of the selectors on the chiral recognition ability were evaluated. The influence of the type and concentration of the organic modifier on the separation of miconazole enantiomers and the pH of the run buffer on the separation of enilconazole enantiomers was also studied.

1. Introduction

Many imidazole derivatives are widely used or recommended for pharmaceutical use as antimycotics (clotrimazole, miconazole, enilconazole, etc.), antineoplastic agents (dacarbazine), antiepileptics (nafimidon, denzimol) cytostatics (erbulozol), thromboxane synthetase inhibitors (dazoxiben), etc. [1]. Some of the pharmaceutically used imidazole derivatives contain a chiral carbon atom and therefore exist as racemic mixtures of the enantiomers. Substantial differences in pharmacokinetic, pharmacodynamic and toxic properties between the enantiomers of many chiral drugs are well established [2,3].

A prerequisite for the accurate study of stereoselective effects of the action of chiral drugs is the development of a versatile and accurate method for the resolution of enantiomers. Capillary electrophoresis (CE) meets this requirement and has been rapidly established as the method of choice for enantiomeric analysis during the last few years [4–13]. The application of new types of chiral selectors with higher efficiency is of primary importance in this area at present.

The chiral resolution of some racemic imidazole and triazole derivatives using CE has been reported recently [5–8], but no systematic study has yet been performed. This study was conducted in order to evaluate systematically the resolution of chiral drugs containing an imidazole moiety by CE. The effects of the concentration of the chiral selector and the organic modifier and the pH of the run buffer on the enantioseparation were studied.

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2. Experimental

2.1. Equipment

A Grom 100 capillary electrophoresis system (Grom, Herrenberg, Germany), equipped with a Linear Instruments (Reno, NV, USA) UVIS 200 detector and a HP 3396 A integrator (Hewlett-Packard, Avondale, PA, USA), was used with an untreated fused-silica capillary (Grom) of total length 61 cm and effective length 44 cm × 50 μm I.D. The electric field was 400 V/cm and the temperature was 21 ± 1°C. The samples were introduced hydrostatically (10 cm) at the anodic end of the capillary during 5 s. The detection of the solutes was carried out at 210 nm. The anode and cathode buffers had the same pH and molarity as the run buffer but contained no chiral selectors.

2.2. Chemicals and reagents

The racemic drugs (Fig. 1) were gifts from the manufacturers. SBE-β-CD (degree of substitution ca. 3.14, $M_r = 1684$) was a kind gift from Professor J.F. Stobaugh and Professor V.J. Stella (Center for Drug Delivery Research, University of Kansas, Lawrence, KS, USA).

α-, β-, γ- and HP-β-CD were obtained from Wacker Chemie (Munich, Germany), analytical-reagent grade KH_2PO_4, Na_2HPO_4, H_3PO_4 and NaOH from Merck (Darmstadt, Germany) and HPLC-grade methanol, acetonitrile and 2-propanol from J.T. Baker (Deventer, Netherlands).

2.3. Preparation of solutions buffer and sample solutions

Stock standard solutions of 50 m*M* KH_2PO_4 and 50 m*M* Na_2HPO_4 were prepared in doubly distilled, deionized water. The pH was adjusted with 0.5 *M* H_3PO_4 or 0.5 *M* NaOH. The run buffers were prepared accordingly after the addition of appropriate amounts of the chiral selectors. All solutions were filtered and degassed by sonication before use. Stock standard solutions of 1 mg/ml of the racemic drugs were prepared, stored at 4°C and diluted to 60 μg/ml before use.

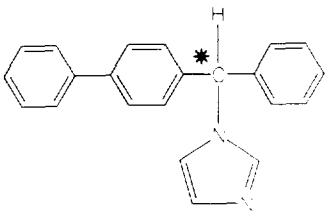
3. Results and discussion

3.1. Effect of the type of chiral selector on chiral recognition

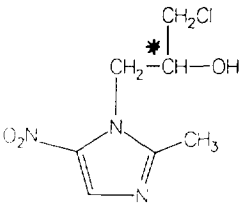
The structures of eight representative members of chiral imidazole derivatives are shown in Fig. 1. The results of the resolution of these derivatives by CE using five native and derivatized cyclodextrins as chiral selectors are summarized in Table 1. The effect of the cavity size is obvious. Apparently α-CD does not bind at least stereoselectively with any of the imidazole derivatives studied. In contrast, the enantiomers of most of them are resolved using γ- and β-CD and also with uncharged (HP-β-CD) and charged (SBE-β-CD) derivatives. HP-β-CD seems to have better chiral recognition abilities than the native cyclodextrins. Thus, enantiomers of miconazole (**4**) and econazole (**5**) could not be resolved using native α-, β- or γ-CD, whereas the enantiomers of these drugs were baseline resolved using HP-β-CD. The chiral selector SBE-β-CD seems to be exceptionally effective and gives baseline separations for most of the racemic compounds in the concentration range 0.1–1.0 m*M* in the run buffer. In Table 1 the decisive role of hydrophobic forces in selectand–selector interactions is demonstrated. In particular, a hydrophobic phenyl moiety is absent in the ornidazole molecule (**2**) and, moreover, a hydrophilic hydroxy group is present. As a result, the enantiomers of this compound could not be resolved using any of the chiral selectors used.

3.2. Effect of concentration of chiral selector on the resolution of enantiomers

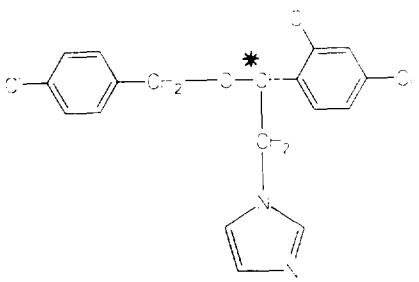
The concentration dependence of the enantiomeric resolution (R_s) was studied with racemic enilconazole (**5**) as an example. It seems noteworthy that nearly the same resolution (R_s) and selectivity (α) were achieved with chiral selector concentrations varying in range 0.07–12.5 m*M* depending on their type (Fig. 2).



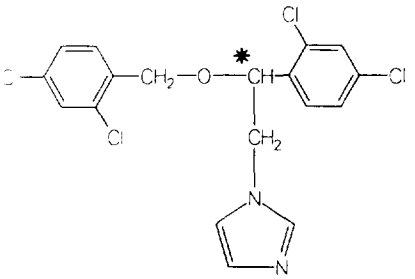
Bifonazole (1)



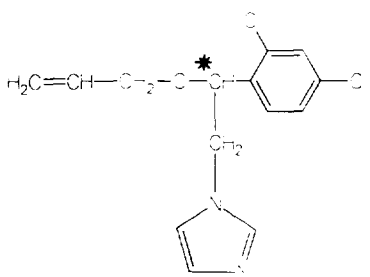
Ornidazole (2)



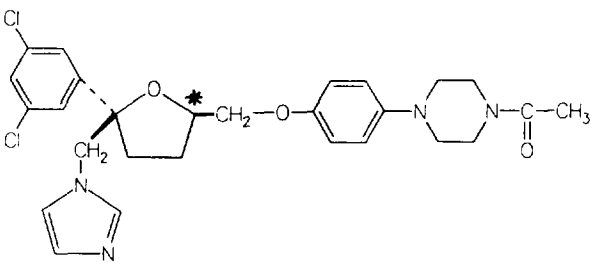
Econazole (3)



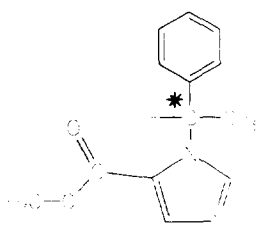
Miconazole (4)



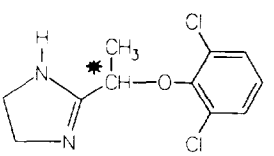
Enilconazole (5)



Ketoconazole (6)



Metomidate (7)



Lofexidine (8)

Fig. 1. Structures of the racemic drugs.

Table 1
Chiral separation of imidazole derivatives

Compound	50 m*M* α-CD–10% MeOH		20 m*M* β-CD–10% MeOH		50 m*M* γ-CD–10% MeOH		20 m*M* HP-β-CD–10% MeOH		0.1 m*M* SBE-β-CD–20% MeOH		1 m*M* SBE-β-CD–0% MeOH	
	t_1/t_2	R_s	t_1/t_2	R_s	t_1/t_2	R_s	t_1/t_2	R_s	t_1/t_2	R_s	t_1/t_2	R_s
Bifonazole	14.86	–[a]	13.59	–[a]	16.95	–[a]	16.90	–[a]	15.20/15.33	1.01	–	
Econazole	16.78	–[a]	15.39	[a]	18.30	–[a]	15.80/16.10	1.60	12.92/13.69	2.46	–	
Enilconazole	14.97	–[a]	11.96/12.30	1.92	12.69/13.16	2.87	12.33/13.19	6.75	7.80/8.10	2.34	–	
Ketoconazole	14.35	–[a]	11.16/11.25	0.61	14.87/14.96	0.67	13.71	–[a]	12.01[b]/12.62[b]	1.66[b]	–	
Lofexidine	9.01	–[a]	10.44/10.59	1.47	11.33/12.01	5.10	9.16/9.40	2.72	6.21	–[a]	8.80/9.09	1.03
Metomidate	10.68	–[a]	9.76/10.17	2.79	13.61/14.46	5.85	12.35/12.81	3.45	8.85	–[a]	9.03/9.26	2.21
Miconazole	17.19	–[a]	15.06	–[a]	20.20	–[a]	15.46/15.74	1.55	12.41/13.07	1.44	–	
Ornidazole	30.07	–[a]	34.00	–[a]	32.00	–[a]	30.80	–[a]	19.17	–[a]	–	

[a] No enantioseparation.
[b] 0.1 m*M* SBE-β-CD–40% MeOH.

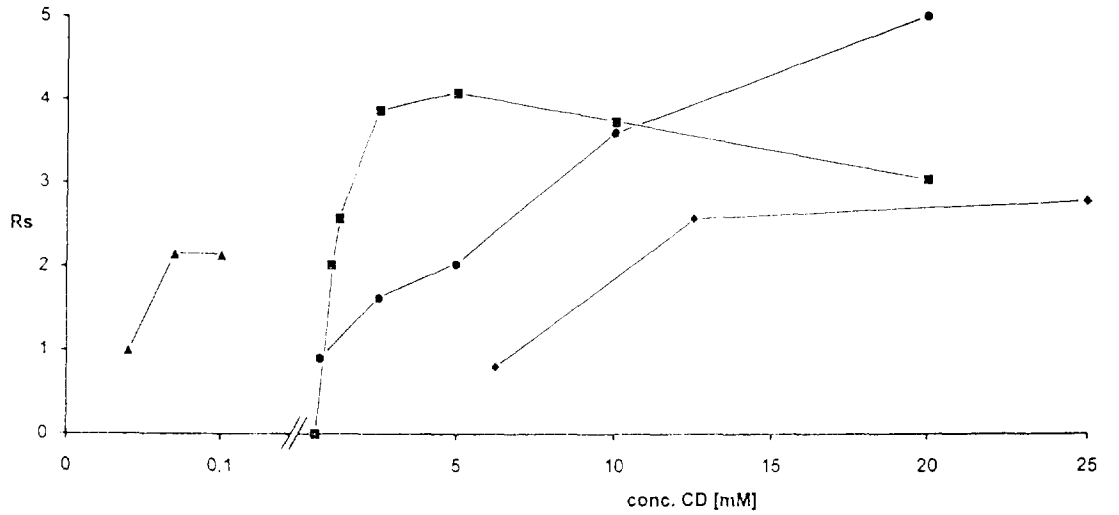


Fig. 2. Plot of resolution (R_s) of enilconazole (**5**) enantiomers vs. concentration of the various cyclodextrins in the run buffer. Conditions: 50 m*M* phosphate buffer (pH 3.0); 400 V/cm; detection at 210 nm. ■ = β-CD; ● = HP-β-CD; ◆ = γ-CD; ▲ = SBE-β-CD.

3.3. pH dependence of the separation of enilconazole

Enilconazole (**5**) seems to be protonated at a pH lower than ca. 6.0–7.0, hence the separation of its enantiomers seems not to be possible at pH > 7 using uncharged cyclodextrins as chiral buffer modifiers. Fig. 3 shows the chiral separation of enilconazole with a run buffer containing various amount of SBE-β-CD and β-CD

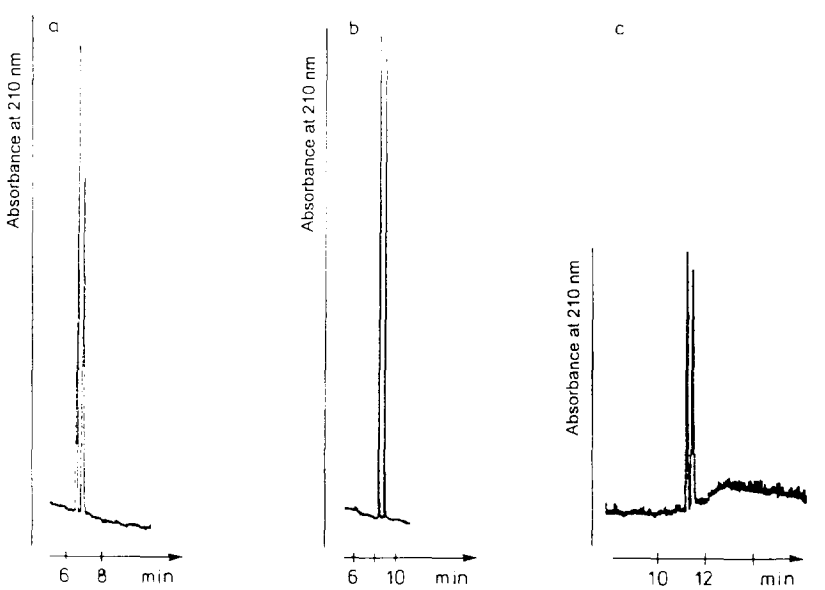


Fig. 3. Separation of enilconazole (**5**) enantiomers. Conditions: 50 m*M* phosphate buffer; detection at 210 nm; (a) pH 3.0, 0.1 m*M* SBE-β-CD–10% methanol, 400 V/cm; (b) pH 3.0, 2.5 m*M* β-CD–10% methanol, 400 V/cm; (c) pH 9.0, 1.0 m*M* SBE-β-CD–10% methanol, 200 V/cm.

in acidic (pH 3.0) and alkaline (pH 9.0) media. Both chiral selectors discriminate between enilconazole enantiomers at pH 3.0, but the amount of SBE-β-CD needed is markedly smaller than that of β-CD. At pH 9.0 a run buffer containing 1 m*M* SBE-β-CD permits adequate enantioseparation, whereas β-CD concentrations as high as 20 m*M* do not show any sign of enantioseparation with buffer of the same pH.

3.4. *Effect of organic modifier on selectivity and peak resolution*

The influence of the concentration of the organic modifier in enantioseparations was also studied. The important role of organic modifiers in CE enantioseparation was first reported by Fanali [4]. It has been considered that the organic modifier can have two roles: (a) improving the solubility of chiral substances and (b) decreasing the affinity of chiral compounds for the hydrophobic cavity of chiral selectors. The former factor diminishes the interaction of substances with the capillary wall and leads to decreased peak broadening whereas the latter needs to be optimized as decreasing the selectand–selector interactions also improves the peak shape but sometimes is accompanied by a substantial loss of selectivity.

Fig. 4 shows the dependence of the peak resolution on the content of organic modifier in the run buffer. There is always one optimum concentration of the organic modifier for chiral resolution. This is to be expected as the result of above-mentioned two opposite effects: increase in efficiency (N) and decrease in selectivity (α) with increase in the concentration of organic modifier. The effect with 2-propanol seems to be most pronounced.

4. Conclusions

The enantiomeric resolution of racemic imidazole derivatives was performed using high-performance CE with various cyclodextrin-type chiral selectors in the run buffer. The effects of the cavity size, the structure and the charge of the chiral selector were studied. The influence of the type and concentration of the organic modifier on the separation of the miconazole enantiomers and of the pH of the run buffer on the separation of enilconazole enantiomers was also investigated. α-CD does not exhibit a chiral recognition ability for the imidazole derivatives studied. HP-

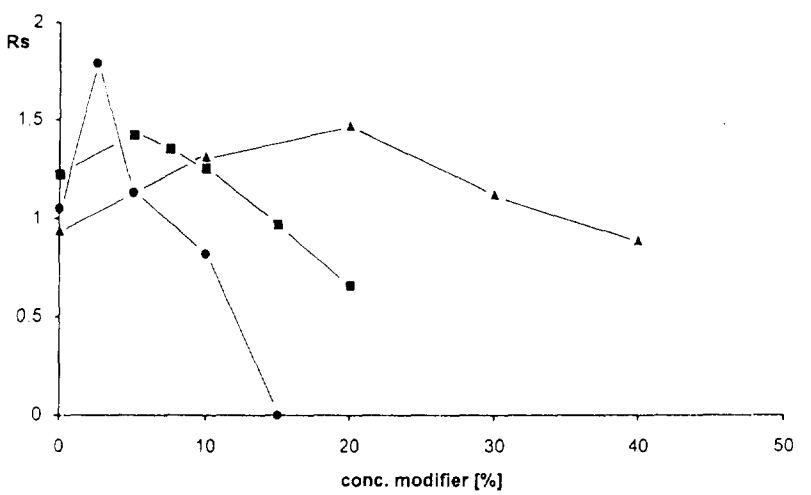


Fig. 4. Plot of resolution (R_s) of miconazole (**4**) enantiomers vs. concentration of various organic modifiers in the run buffer. Conditions: 50 m*M* phosphate buffer (pH 3.0); 0.1 m*M* SBE-β-CD; 400 V/cm; detection at 210 nm. ▲ = Methanol; ■ = acetonitrile; ● = 2-propanol.

β-CD shows better chiral recognition ability than the native CDs. The negatively charged SBE-β-CD seems to be the most efficient chiral selector.

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