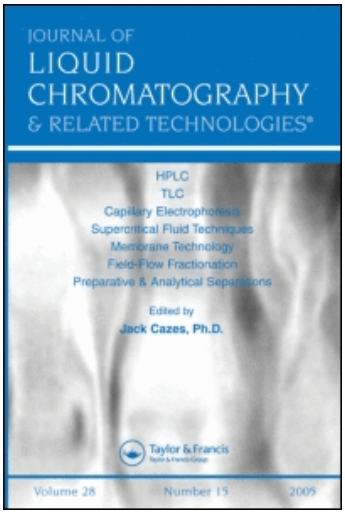

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CHIRAL SEPARATION OF REDUCED HALOPERIDOL BY CAPILLARY ZONE ELECTROPHORESIS WITH HEPTAKIS (2,6-DI-O-METHYL)-β-CYCLODEXTRIN

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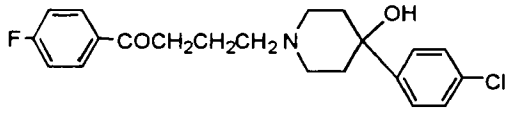
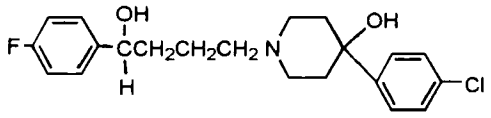
ABSTRACT

A simple capillary zone electrophoresis was developed for the chiral separation of reduced haloperidol, a chiral metabolite of antipsychotic drug, haloperidol. Reduced haloperidol was separated in a phosphate buffer (pH 2.5) using heptakis (2,6-di-O-methyl)-β-cyclodextrin as a chiral selector. Several parameters such as type of cyclodextrin, pH and concentration of buffer, applied voltage, concentration of chiral selector and detection wavelength that affected the chiral separation and detection of reduced haloperidol were discussed.



(HP)



(RHP)

Figure 1. Chemical structures of haloperidol (HP) and reduced haloperidol (RHP).

INTRODUCTION

Haloperidol (HP) is one of the most widely used drugs for the treatment of schizophrenia and related psychotic disorders. After administration of HP, it is partly biotransformed to reduced haloperidol (RHP), a chiral metabolite as shown in Fig. 1.

Monitoring the plasma levels of both HP and RHP has been suggested as a better therapeutic indicator for patients undergoing HP treatment, but there are inconsistent reports[1-4] that a higher RHP to HP ratio in plasma is good or bad for therapeutic response. Since RHP is an optically active species, the enantiomeric composition of RHP and its possible role in HP therapy is of great interest.

A variety of liquid chromatographic (LC) methods[5-30] have been used for the analysis of HP/RHP in various samples, but only limited method[31] demonstrated the separation of synthetic RHP by LC with chiral column. In this report a preliminary work on the chiral separation of RHP by capillary electrophoresis (CE) with neutral cyclodextrin was performed.

The results indicated that the enantiomers of RHP can be simply resolved by CE in acidic phosphate buffer with heptakis (2,6-di-O-methyl)-β-cyclodextrin (dimethyl-β-CD) as a chiral selector. This may be a favorable step to investigate the chiral profile of RHP in HP therapy.

METHODS

Apparatus

A Beckman P/ACE system 2000 (CA, USA) equipped with a filtered UV detector and with a liquid-cooling device was used. Capillary zone electrophoresis was performed in a neutral coated capillary (Beckman) of 37 cm X 50 μm I.D. (effective length, 30 cm). Samples were injected by pressure for 1 s, equivalent to a volume of about 1.78 nL (according to the specification of the manufacturer) and the applied voltage was 22 kV. Separations were achieved at about 21°C with phosphate buffer (40 mM, pH 2.5) including dimethyl-β-cyclodextrin (10mM). The above-mentioned CE conditions were used for the general experiment unless stated otherwise.

Chemicals and Solutions

RHP (Janssen, Olen, Belgium), HP (Aldrich, WI, USA), dimethyl-β-cyclodextrin (dimethyl-β-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD) and hydroxypropyl β-cyclodextrin (HP-β-CD) (Beckman), α-cyclodextrin (α-CD), phosphoric acid (H_3PO_4) and disodium hydrogen phosphate (Na_2HPO_4) (Nacalai, Osaka, Japan), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), 6-O-α-maltosyl-α-CD, sodium dihydrogen phosphate (NaH_2PO_4) and hydrochloric acid (Wako, Osaka, Japan) were used without further treatment. Milli-Q (Millipore, CA, USA) treated water was used for the preparation of buffer and related aqueous solutions.

Solutions of various phosphate buffers at pH 2.5 or 4.5 were obtained by neutralizing related NaH_2PO_4 solution with H_3PO_4 solution, each at same concentration; solutions of phosphate buffer at pH 6.5 were prepared by neutralizing Na_2HPO_4 solutions with H_3PO_4 solution, each at same concentration. Stock solutions of RHP (1.0 mM) and HP (1.0 mM) were prepared in 0.1 M HCl and suitably diluted with phosphate buffer (40 mM, pH 2.5) as working solution.

RESULTS AND DISCUSSION

Optimal parameters for the chiral separation and detection of RHP at 66 μM were studied, including the wavelength for absorption, the neutral CD as chiral selector, pH and concentration of phosphate buffer, applied voltage and concentration of dimethyl-β-CD. It was found simple in the present study to

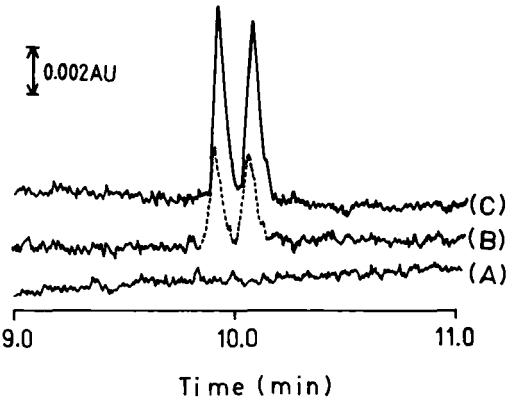


Figure 2. Composite electropherograms of RHP detected at (A) 254 nm, (B) 214 nm and (C) 200 nm. Conditions: buffer, 40 mM phosphate (pH 2.5) with 10 mM dimethyl-β-CD; capillary electrophoresis at 22 kV; sample, RHP at 66 mM; see text for other conditions.

use the resolution[32] (R) stated below for screening the parameters for the separation of RHP, i.e., $R = (h_m - h_V) / h_m$, where h_m is the mean peak height and h_V is the height of the valley. This gives the values of R between 0 and 1 (as baseline resolution).

Effect of Wavelength for Detection

After chiral separation of RHP in phosphate buffer (40 mM, pH 2.5) with 10 mM dimethyl-β-CD as a chiral selector, RHP was monitored at 200, 214 or 254 nm. The results in Fig. 2 indicated that a higher response can be obtained with RHP detected at 200 nm, due mainly to a larger molar absorptivity obtained from favorable excitation of RHP at the lower UV irradiation.

Effect of Neutral CD

Several neutral CDs were tested as chiral selector for the separation of RHP in phosphate buffer (40 mM, pH 2.5) at 22 kV. The results in Table 1 indicated that the enantiomeric resolution of RHP is better when dimethyl-β-CD, α-CD or 6-O-α-maltosyl-α-CD was used as a chiral selector. Dimethyl-β-

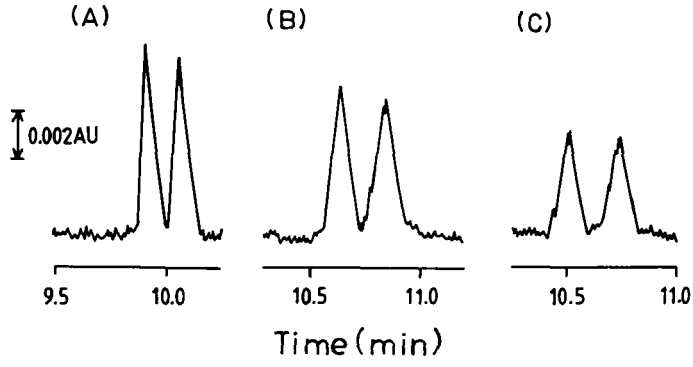


Figure 3. Electropherograms of RHP separated with (A) dimethyl-β-CD (10 mM), (B) α-CD (10 mM) and (C) 6-O-α-maltosyl-α-CD (10 mM). See text for conditions.

Table 1

Resolution Values for RHP Enantiomers Separated with Neutral Cyclodextrins

Cyclodextrin	Resolution*	
	5mM	10mM
α-CD	0.86	0.97
β-CD	0	0
γ-CD	0	0
DM-β-CD	0.81	0.99
HP-β-CD	0.12	0.45
TM-β-CD	0	0.40
6-O-α-Maltosyl-α-CD	0.91	0.97

* Resolution (R) of RHP in phosphate buffer (40 mM, pH 2.5) with cyclodextrins each at 5 and 10mM; R, expressed as $(h_m=h_v)h_m$, where h_m for the mean peak height and h_v for the height of the valley. See text for conditions.

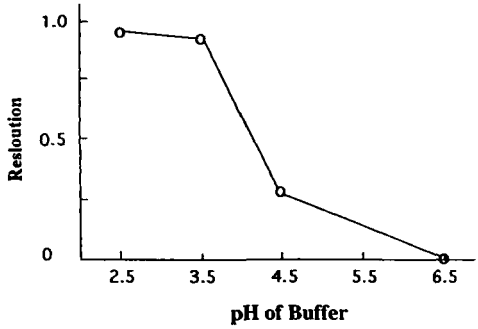


Figure 4. Effect of the pH of phosphate buffer (40 mM) on the resolution of RHP. See text for conditions.

CD was selected as the chiral additive in phosphate buffer for the separation of RHP, because it gave sharper peaks of RHP as shown in Fig. 3 . It is interesting to observe that α-CD and its derivative are also able to recognize the enantiomers of RHP. This reveals that the chiral moiety of RHP including the lipophilic 4-fluorophenyl group may do good fit in the cavity of α-CD.

The potential use of α-CD as a chiral selector for RHP seems to be very attractive.

Effect of pH of the Buffer

Fig. 4 indicated that chiral separation of RHP at higher pH resulted in poor resolution of the enantiomers of RHP. As a consequence, separation of RHP in phosphate buffer (40 mM) at pH 2.5 was performed.

Effect of the Applied Voltage

Resolution of RHP at a voltage range of 14-26 kV was studied. The results in Fig. 5 indicated that there are no apparent change of resolution (an R range of 0.93-0.98) except that a higher applied voltage definitely led to a shorter migration time (t_m) of RHP (a t_m range of about 8.6 to 16.3 min).

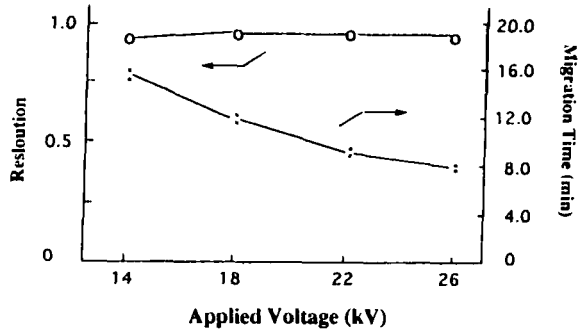


Figure 5. Effect of applied voltage on the resolution and migration of RHP. See text for conditions.

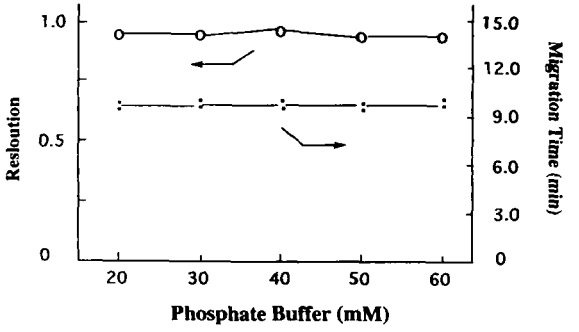


Figure 6. Effect of buffer's concentration on the resolution and migration of RHP. See text for conditions.

Effect of the Buffer Concentration

Chiral separation of RHP in phosphate buffer (pH 2.5) at concentration range of 20-60 mM with constant dimethyl-β-CD (10 mM) was examined. The results in Fig. 6 showed that there are no apparent change of resolution (an R range between 0.94-0.98) and migration time (a t_m range of about 9.6-10.0). Increasing the concentration of the buffer usually results in a higher viscosity of

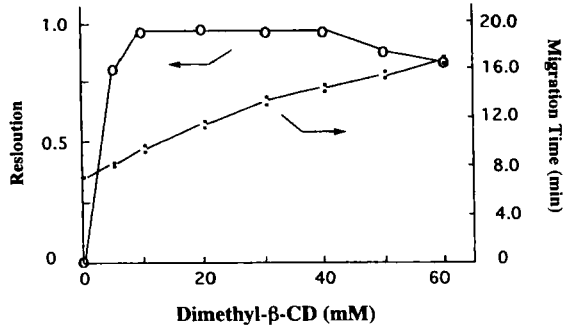


Figure 7. Effect of the concentration of chiral selector on the resolution and migration of RHP.

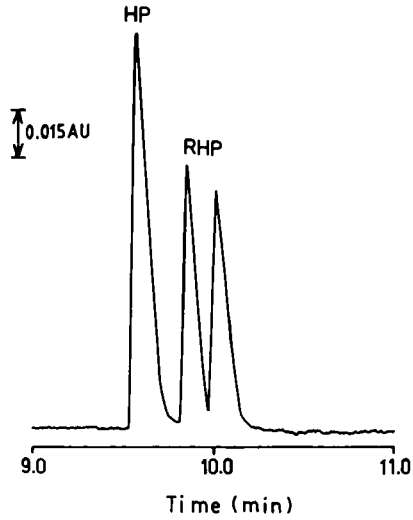


Figure 8. Electropherogram for a standard mixture of HP (0.27 mM) and RHP (0.26 mM). See text for conditions.

the buffer, in turn, leads to a larger Joule's heat in electrophoresis that interactively offsets the viscosity effect. This may partially explain that only minute variation of migration time was observed in CE of RHP at elevated buffer concentrations.

Effect of the Concentration of Dimethyl-β-CD

Optimal concentration of a chiral selector may exist for the resolution of certain enantiomers.[33] Therefore, the selected chiral selector, dimethyl-β-CD, at concentrations between 0-60 mM in phosphate buffer (40 mM, pH 2.5) was investigated for the separation of RHP. The results in Fig. 7 indicated that the concentrations of dimethyl-β-CD at 10-30 mM gave better resolution of RHP. A typical electropherogram for a standard mixture of RHP and HP was illustrated in Fig. 8, indicating the minimally acceptable resolution of RHP enantiomers and the full separation of RHP from its parent drug, HP.

In conclusion, a simple capillary zone electrophoresis is established for the chiral separation of RHP in phosphate buffer (40 mM, pH 2.5) using dimethyl-β-CD (10 mM) as the chiral selector. Coupled with a preconcentration treatment of RHP from biological sample, the present CE method seems to be a potential approach to study the chiral profile of RHP from patients undergoing HP therapy; namely, the metabolic form of RHP appears as an enantiomer, a racemate or other enantiomer mixture can be investigated.

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