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Chiral Separation of β-Blockers after Derivatization with (-)-Menthyl Chloroformate by Reversed-Phase High Performance Liquid Chromatography

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Optimum conditions of chiral derivatization reaction of β-blockers(acebutolol, arotinolol, betaxolol, bisoprolol, celiprolol, metoprolol and pindolol) with (-)-menthyl chloroformate were investigated for the resolution by HPLC. With more than 30 times molar excess of (-)-menthyl chloroformate chiral derivatization reactions were completed within one hour at room temperature except arotinolol and celiprolol. Diastereomeric derivatives of β-blockers were well resolved on the ODS column using acetonitrile-methanol-water as a mobile phase.

Key words: Chiral derivatization, β-Blocker, Resolution, (-)-Menthyl chloroformate

INTRODUCTION

β-adrenoceptor blocking agent is used in the treatment of hypertension, angina pectoris and arrhythmias (Mehvar *et al.*, 1989). β-blocking drug sustances most often exhibit a chiral structure and are mostly used as racemic mixtures (Ekelund *et al.*, 1995). The pharmacology activity of these drugs, however, resides predominantly in their S-enantiomer and has some side effects related to R-enantiomer(Nathanson *et al.*, 1988). Therefore when drug is used clinically as a racemic mixture, it is administered as two different drugs with different pharmacokinetic and potentially pharmacodynamic properties. The great difference in pharmacological effect and pharmacokinetics between the two enantiomeric forms has needed methods for enantioselective separation.

The classical method for the determination of optical purity, also in pharmacopoeial monographs of drug sustances, is optical rotation. However, this method is neither very accurate nor precise, and it will be difficult to control the content of enantiomeric impurity at low levels. In recent years, HPLC techniques that are capable of separating and determining enantiomers have been greatly improved (Ekelund *et al.*, 1995). Chromatographic separations of enantiomers can either be carried out by a indirect method, witch involves the formation of diastereomeric pairs by using chiral derivatization agents or directly by using a chiral stationary phase or chiral additives to the mobile phase. The use of chiral stationary phase on β-blockers has been reported such as the pirkle type, α_1-acid glycoprotein and β-cyclodextrin (Nakamura *et al.*, 1995, Veigl *et al.*, 1995, Christopher *et al.*, 1995, Ceccato *et al.*, 1997, Huynh *et al.*, 1995, Chassaing *et al.*, 1996 and Hermansson *et al.*, 1995). But the results obtained seem less promising owing to low separation factors or considerable peak broadening. Indirect method has a disadvantage including derivatization procedure but has a advantage of improved peak symmetry and resolution since the separation occurs on achiral column (Srinivas *et al.*, 1992). Several examples of separating β-blockers following chiral derivatization have been reported such as (S)-(-)- or (R)-(+)-1-phenylethyl isocyanate (Pflugmann *et al.*, 1987), (R)-(-)-1-(-1-naphthyl) ethyl isocyanate (Bhatti *et al.*,1992), 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (Schuster *et al.*, 1988) and (-)-menthyl chloroformate (Mehvar *et al.*, 1988 and Li *et al.*, 1995). No publications so far have investigated the optimum conditions of derivatization reaction and only a few have discussed the suitability of determining optical purity and separation power.

In this paper, eight β-blockers currently on sale(Fig. 1) were investigated for enantiomeric separation using (-)-menthyl chloroformate, which is possibly the lowest-priced and available commercially (Schmitthenner *et al.*,

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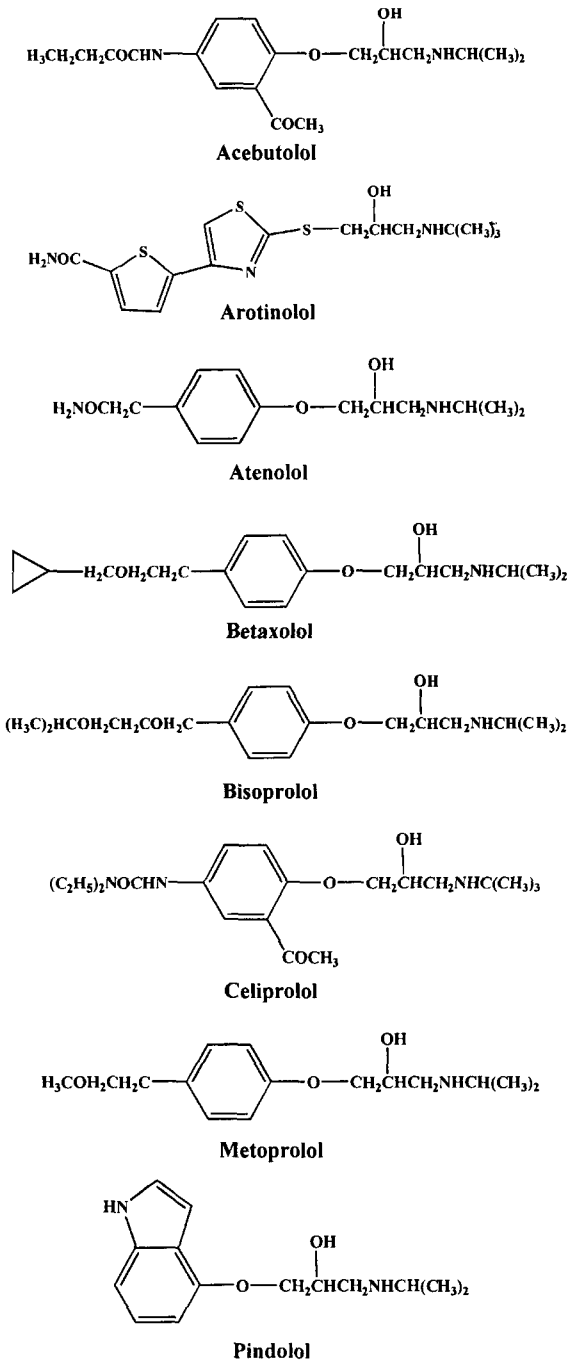

Fig. 1. Structures of β-blockers

1989 and Witte *et al.*, 1991). The effects of reaction time, reaction temperature and concentration of (-)-menthyl chloroformate on derivatization of β-blockers were investigated and optimum conditions of derivatization could be applied to resolution of β-blockers.

MATERIALS AND METHODS

Materials and equipment

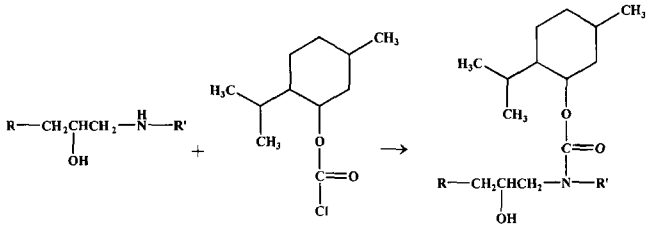

Fig. 2. Derivatization reaction of β-blockers using (-)-MCF.

Atenolol and pindolol hydrochloride were obtained from Il Dong Co. (Seoul, korea), acebutolol hydrochloride from Rhone-Poluenc Rorer Korea (Seoul, Korea), arotinolol hydrochloride from Cheil Jedang (Seoul, Korea), betaxolol hydrochloride from Bu Kwang Co. (Seoul, Korea), bisoprolol hemifumarate from Yuhan Cyanamide (Kunpo, kyeonggi, Korea), celiprolol hydrochloride from Young Jin Co. (Seoul, Korea) and metoprolol tartarate from Yuhan Co. (Seoul, Korea). Molecular structures are shown in Fig. 1. (S)-(-)-menthyl chloroformate((-)-MCF) was purchased from Tokyo Organic chemicals (Tokyo, Japan). *Trans*-4-hydroxy-L-proline was purchased from sigma (St. Mouis, Mo, USA). Methanol and acetonitrile as a HPLC grade and other reagents as a analytical grade were obtained from Duksan Pure Chemicals Co. (Ansan, Kyeonggi, Korea).

The chromatographic systems consisted of PU 610 pump, UV 620 UV/VIS detector with variable wavelengths (GL science, Tokyo, Japan) and Rheodyne 7725i injector with a 20 μl loop. The acqisition of chromatogram and integration used a EZchrom data system (Scientific Software Inc., USA).

Derivatization procedures

Stock solutions of the various substrates (1 mg/ml) were prepared in acetonitrile. Aliquots of these solutions (100 μl) were pipetted into a screw-capped tube, evaporated to dryness under a stream of nitrogen, and the residues were dissolved in 400 μl of (-)-MCF solution (1.12 mM, in acetonitrile). The solution was vortex mixed and kept at room temperature for 1 h. After incubation, 200 μl of proline solution (2.23 mM, in saturated Na_2CO_3 solution) was added immediately to quench the reaction and then the reaction tube was centrifuged for 5 minutes at 3000 rpm. A aliquot of the acetonitrile layer (20 μl) was injected directly on to the column.(fig. 2)

Chromatography

The HPLC separation of the diastereomeric products was performed using a reverse phase system. The chromatographic column used were Inertsil ODS-2 (150 × 4.6 mm I.D., GL science, Tokyo, Japan). The mobile phases were acetonitrile-methanol-water with various composition at a flow-rate 1 ml/min. Further details are given

Table I. Chromatographic data for (-)-MCF derivatized β-blockers

β-blockers	λ(nm)	Mobile phase	Chromatographic parameters			
		MeCN-MeOH-Water	k_1'	k_2'	α	Rs
Acebutolol	327	0 : 78 : 22	5.11	5.97	1.17	2.06
Arotinolol	317	0 : 90 : 10	8.47	9.28	1.10	1.16
Atenolol	276	0 : 73 : 27	7.28	8.29	1.14	1.64
Betaxolol	276	60 : 24 : 16[a]	10.08	10.78	1.07	1.20
Bisoprolol	273	0 : 80 : 20[b]	9.06	9.87	1.03	1.07
Celiprolol	330	0 : 79 : 21	6.36	7.00	1.10	1.18
Metoprolol	276	0 : 80 : 20	8.66	9.46	1.10	1.18
Pindolol	265	0 : 77 : 23	5.80	6.51	1.12	1.31

in Table I. In order to screen the chromatographic properties of the derivatives, UV detection with variable wavelength (273~330 nm) was employed.

Optimization of derivatization of β-blockers with (-)-MCF

A solutions of the compounds(100 μl, equivalent to 100 μg) was pipetted into a glass tube and evaporated to dryness and treated in the same way as described above. The whole was incubated at room temperature, 50°C, 70°C and reaction was quenched adding 200 μl of proline solution (2.23 mM, in saturated Na_2CO_3 solution) given reaction time from 15 seconds to 3 h. After centrifugation, a aliquot of the acetonitrile layer was injected directly on to the column.

A solutions of the compounds (100 μl, equivalent to 100 μg) was placed in screw-capped tube and was added 400 μl of (-)-MCF solution at various molar ratio, from 2-fold molar excess to 100-fold molar excess. Reaction mixtures were analyzed in the same way as described above.

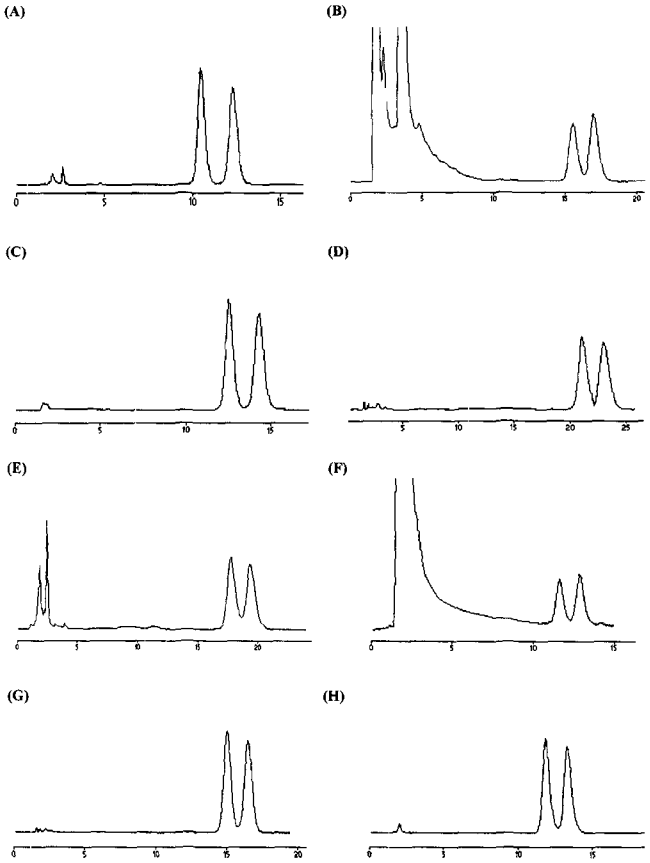

Fig. 3. Chromatograms of the diastereomers obtained from racemic β-blockers after derivatization with (-)-MCF; detailed chromatographic conditions see Table I.
(A) Acebutolol (B) Arotinolol (C) Atenolol (D) Betaxolol (E) Bisoprolol (F) celiprolol (G) Metoprolol (H) Pindolol

RESULTS AND DISCUSSION

Chromatographic behaviour of the derivatives

(-)-Menthyl chloroformate reacted selectively with β-blockers to form the corresponding diastereomeric carbamate. These diastereomers were well separable by RP-HPLC. Fig. 3 shows chromatograms of β-blockers derivatized with (-)-MCF. A detailed presentation, giving capacity factors (k'), separation factors(α) and resolution (Rs) of the chromatographic results obtained by the above mentioned procedures is shown in Table I. The α-values, whitch ranged from 1.03 to 1.17, and peak resolution values (Rs), ranging from 1.07 to 2.06, were obtained with k' below 10 (except for betaxolol with k' value of 10.08 and 10.78). The best resolution was obtained for acebutolol (Rs 2.06) and it appears that with the exception of bisoprolol all the β-blockers could be baseline separated with k' value below 11 as (-)-MCF derivatives.

The effects of reaction time and temperature

Reactivity of β-blockers monitored at room temperature, 50°C and 70°C. Reaction time also was monitored from 15 seconds to 3 h. Results are shown in Fig. 4. As the reaction temperature was increased from room

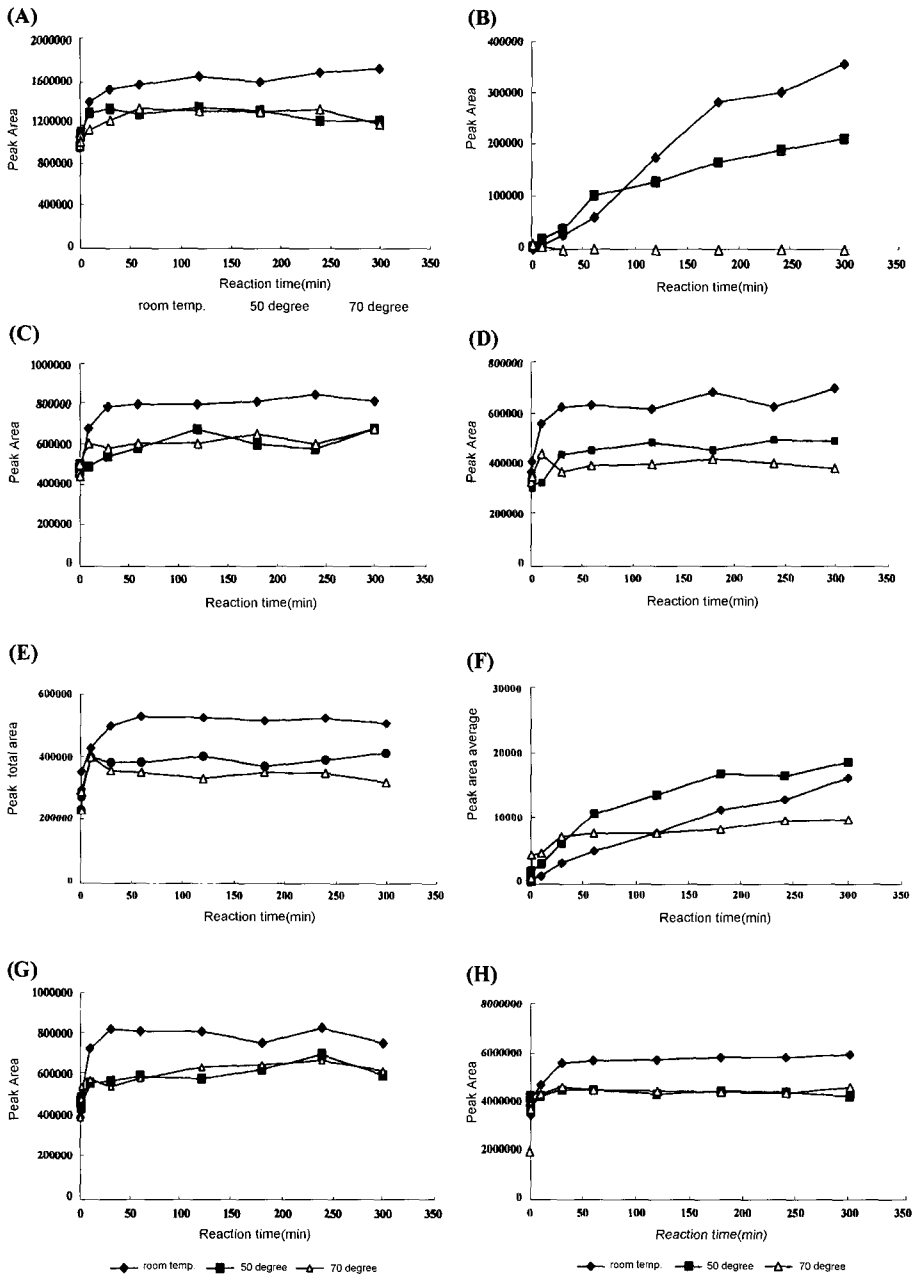

Fig. 4. The effects of reaction time and temperature on derivatization of β-blockers with (-)-MCF. (A) Acebutolol (B) Arotinolol (C) Atenolol (D) Betaxolol (E) Bisoprolol (F) celiprolol (G) Metoprolol (H) Pindolol

temperature to 50°C or 70°C, the exception of celiprolol the peak areas of diastereomers were decreased. In the course of a time study, it was demonstrated that a derivatization of β-blockers except arotinolol and celiprolol was achieved within 60 minutes. The reaction time profile was very similar for six β-blockers (acebutolol, atenolol, betaxolol, bisoprolol, metoprolol and pindolol) and atenolol, betaxolol, bisoprolol, metoprolol and pindolol reacted somewhat faster (completion within 30 minutes). In the arotinolol and celiprolol, the formation rate of diastereomer was increased in proportion to reaction time.

As a interesting effect was noticed in the derivatization of different β-blockers, namely that the reaction time increased with increase in size of alkyl group at the derivatizable amino function. This phenomenon therefore can be interpreted as a steric hindrance effect at the amine site. This steric hindrance may interefere with the chloroformate attack, thus making the derivatization reaction slowly.

The effect of concentration of (-)-MCF

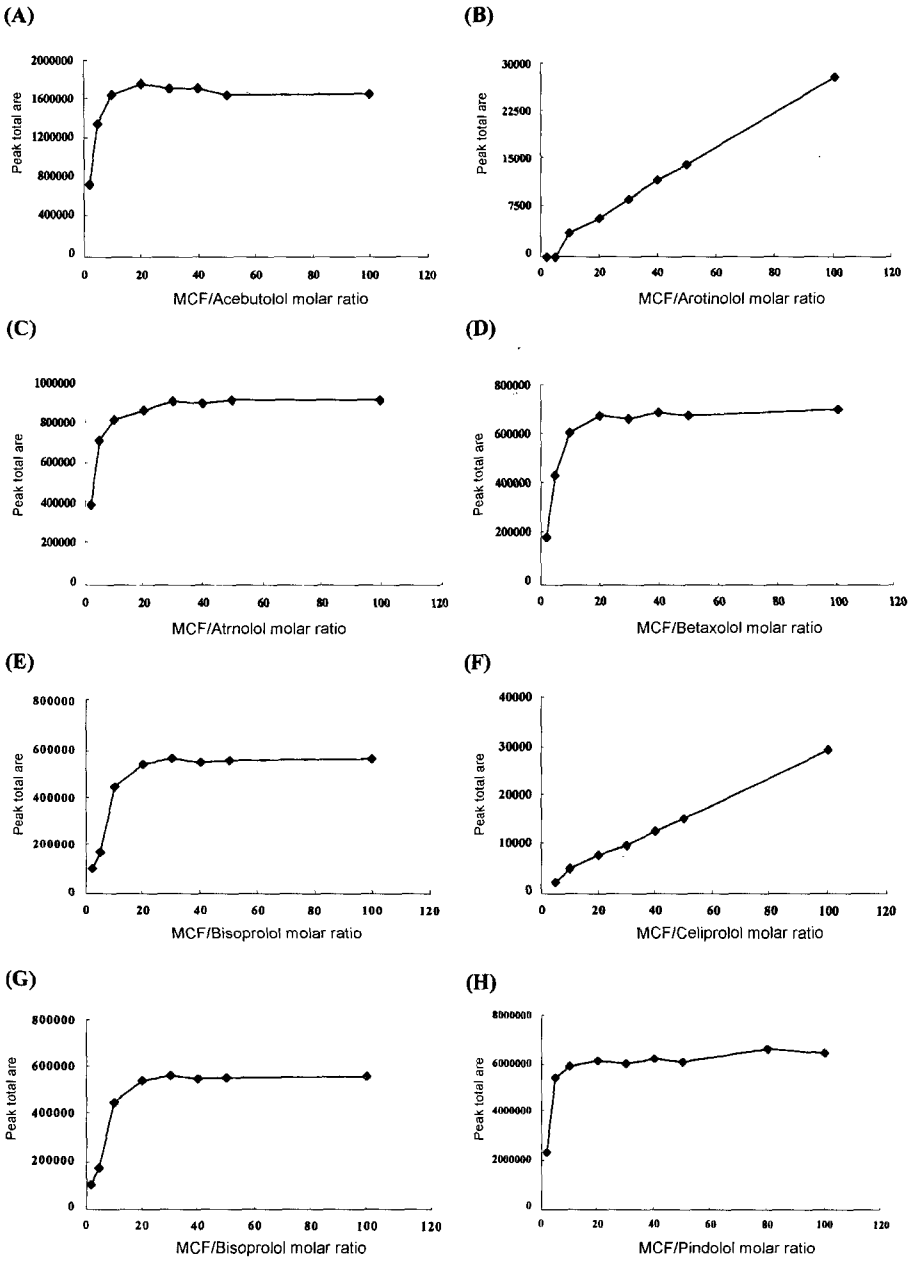

Fig. 5. The effect of concentration of (-)-MCF on derivatization reaction. (A) Acebutolol (B) Arotinolol (C) Atenolol (D) Betaxolol (E) Bisoprolol (F) celiprolol (G) Metoprolol (H) Pindolol

The effect of the concentration of (-)-MCF added was shown in Fig. 5. An increase of the concentration of (-)-MCF led to a general increase in formation of the diastereomers. Among the β-blockers studied, acebutolol, betaxolol and pindolol were showed increase of peak areas of diastereomers upto 20 times molar excess of (-)-MCF and reached a plateau. Derivatives of atenolol, bisoprolol and metoprolol were increased upto 30 times molar excess of (-)-MCF and reached a plateau. However, as described above, reaction of arotinolol and celiprolol increased with increase in molar ratio of (-)-MCF added.

CONCLUSION

Derivatization of eight β-blockers studied were shown some difference optimum conditions. Optimum conditions of derivatization of betaxolol and pindolol were at room temperature for 30 minutes, when 20-fold molar excess of reagent was used. And optimun conditions of derivatization of atenolol and metoprolol were at room temperature for 30 minutes using 30-fold molar

excess of (-)-MCF. Acebutolol and bisoprolol reacted somewhat slower. Then these maximum formation of diastereomers was seen at the reaction time of 60 minutes, when 20-fold and 30-fold molar excess of (-)-MCF was used, respectively. arotinolol and celiprolol with a tertiary butyl group as substituent at the amino function were reacted contiuously with increase in reaction time and the concentration of (-)-MCF and were not reacted quantitatively.

This chemically selective chiral derivatization agent is excellently suited for the indirect resolution of secondary amines using RP-HPLC. Optimum conditions of derivatization reaction investigated could be used determination of optical purity of six β-blockers (except for arotinolol and celiprolol)

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