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Chiral Purity Test of Metoprolol Enantiomer After Derivatization with (-)-Menthyl Chloroformate by Reversed-Phase High Performance Liquid Chromatography

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A reversed-phase high-performance liquid chromatographic method was developed to determine the optical purity of metoprolol enantiomers. The enantiomers were converted to diastereomeric derivatives using (–)-menthyl chloroformate reagent. Separation of the enantiomers as diastereomers was achieved by reversed-phase HPLC within 30min using Inertsil C8 column. This method allowed determination of 0.05% of either enantiomer in the presence of its stereoisomer and method validation showed adequate linearity over the required range. Owing to the reaction condition during the derivatization with (-)-menthyl chloroformate, the possibility of racemization had to be established. Different ratios of (S)-(–)-metoprolol and (R)-(+)-metoprolol were prepared. Enantiomeric separation of these mixtures took place on a chiralcel OD column or, after derivatization with (-)-menthyl chloroformate, on a C8 column. The results from the these two independent separation systems were compared with trace racemization and were in very good agreement. No racemization was found during the experiment.

Key words: Chiral separation, (-)-Menthylchloroformate, Metoprolol, Optical purity, Racemization

INTRODUCTION

The enantiomers of different drugs, which one or multiful asymmetric centres, may differ widely in their biological activities and toxicological properties and it is therefore important to establish methods for the enantiomeric purity testing of chiral drugs (Nishi *et al.*, 1991).

Metoprolol is a β_1 selective aryloxypropanolamine adrenergic antagonist used extensively in the treatment of a variety of cardiovascular disorders and administered as a racemic mixture. (S)-(–)-metoprolol has been reported to be significantly greater β_1-adrenergic receptor affinity by >25-fold than (R)-(+)-metoprolol (Nathanson *et al.*, 1988 and Murthy *et al.*, 1990). A method for the determination of the optical purity of metoprolol and that in preparations such as tablets and injections is required in order to certify the quality. High performance liquid chromatographic methods have been widely used to separation and quantify the enantiomers of compounds in mixtures. Chromatographic separations of enantiomers can either be carried out by a indirect method, which 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 metoprolol has been reported such as the pirkle type (Balmer *et al.*, 1992 and Ekelund *et al.*, 1995), α_1-acid glycoprotein (Enquist *et al.*, 1990 and Hermansson *et al.*, 1995) and β-cyclodextrin (Tran *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 metoprolol following chiral derivatization have been reported such as (R,R)-O,O-diacetyl tartaric acid anhydride (Linder *et al.*, 1984), 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (Schuster *et al.*, 1988), (S)-(+)-1-(1-naphthyl)-ethyl isothiocyanate (Bhatti *et al.*, 1992) and (–)-menthyl chloroformate (Li *et al.*, 1995).

(–)-Menthyl chloroformate, which can react with a hydroxy or an amine group, offers several advantage in

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the resolution of aryloxypropanolamines. The derivatization reaction is facile and provides the stable carbamate diastereomers which may be analyzed directly. The reagent is readily available commercially and is possibly the lowest-priced CDA available. It is derived from (–)-menthol which is isolated from natural source in 100% optical purity. The (–)-menthol moiety possesses three chiral centers in the cyclohexane ring and as such is a rigid. Furthermore, in reversed-phase chromatography the non-bonding, lipophilic interactions are predominant, and recognition of the highly liphophilic (–)-menthol moiety is taken advantage of and the separation of (–)-menthyl carbamates is thus facilitated (Schmitthnner *et al.*, 1989).

This paper describes the reversed-phase HPLC chiral separation of metoprolol using (–)-menthyl chloroformate. Method validation data is examined to determine optical purity for drugs containing metoprolol. Racemization, due to the applied reaction conditions, also was checked for the metoprolol by comparing the results of derivatization with the results of an independent direct separation system.

MATERIALS AND METHODS

Materials and equipment

The tartarate salt of metoprolol was provided by Yuhan corporation (Kunpo, kyeonggi, Korea). (–)-Menthyl chloroformate ((–)-MCF) was purchased from Tokyo Organic Chemicals (Tokyo, Japan) and *trans*-4-hydroxy-L-proline from Sigma (St. mouis, Mo, USA). n-Hexane, ethanol, 2-propanol and methanol as a HPLC grade were obtained from Duksan Pure Chemicals Co. (Ansan, Kyeonggi, Korea). Diethylamine as a analytical grade was obtained from Jusei (Tokyo, Japan).

The chromatographic system consisted of LC-9A pump (Shimadzu, Kyoto, Japan), RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan) with excitation/emission wavelengths of 276/309 nm and Rheodyne 7725*i* injector with a 20 μl loop. The acquisition of chromatogram and integration was carried out with a C-R4A integrator (Shimadzu, Kyoto, Japan). A VG-Trio 2000 mass spectrometer (VG inc., England) was operated in the beam electron impact mode at 70 eV.

Chromatography

For the direct chiral separations, the chromatographic column used was Chiralcel OD (250 × 4.6 mm I.D., Daicel, Tokyo, Japan). The mobile phase was n-hexane-ethanol-2-propanoldiethylamine (88/6/6/0.25, v/v) at a flow rate 1.0 ml/min.

The HPLC separation of the diastereomers formed during derivatization was performed using a reversed-phase system. The chromatographic column used was Inertsil C8 (150 × 4.6 mm I.D., GL science, Tokyo, Japan) and the mobile phase was 73% methanol in water at a flow rate 1.0 ml/min.

Derivatization procedures

Aliquots of a 0.4 mg/ml solution of metoprolol in acetonitrile (100 μl) were pipetted into a 4 ml vial, evaporated to dryness under a stream of nitrogen, and the residues were dissolved in 400 μl of (–)-MCF solution (2.55 M, in acetonitrile). The solution was vortex mixed and kept at room temperature for 30 minutes. After incubation, 200 μl of *trans*-4-hydroxy-L-proline solution (5.10 M, in saturated Na_2CO_3 solution) was added immediatly to quench the reaction and then the reaction tube was centrifuged for 5 minutes at 3000 rpm. A 20 μl of acetonitrile layer was injected into the RP-HPLC system.

Sample preparation

Stock solutions of (S)-(–)- and (R)-(+)-metoprolol were prepared in acetonitrile (0.4 mg/ml). A working solutions were diluted to volume with acetonitrile. Solutions of (S)-(–)-metoprolol and (R)-(+)-metoprolol mixed to give samples with various ratios of the (S)-(–)- and (R)-(+)-enantiomers(range, 0~100% of (S)-(–)-metoprolol in the (R)-(+)-metoprolol). Each mixture was divided into two. One was injected onto the Chiralcel OD column and the other was derivatized in the same way as described above, then was injected into the RP-HPLC system.

RESULTS AND DISCUSSION

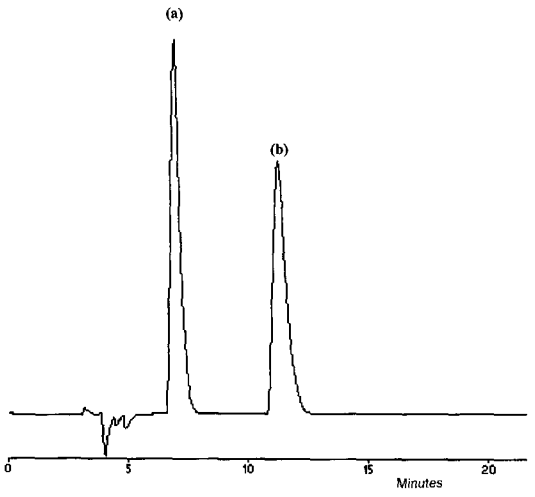

Fig. 1. Chiral semi-preparative HPLC chromatogram of metoprolol enantiomers. Column[Chiralcel OD, 250 × 10 mm I.D.; mobile phase, n-hexane-ethanol-2-propanol-diethylamine (90/5/5/0.25, v/v); detector, UV 276 nm]. Peak a, (R)-(+)-metoprolol; peak b, (S)-(–)-metoprolol.

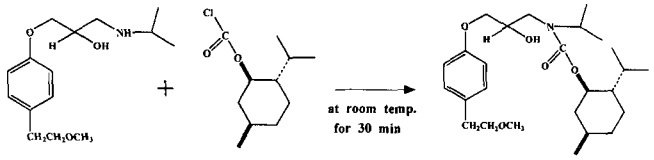

Fig. 2. Derivatization reaction of metoprolol with (-)-menthyl chloroformate.

Chiral semi-preparative HPLC of (R)-(+)-metoprolol and (S)-(-)-metoprolol

Metoprolol tartarate 100 mg was dissolved in 10 ml of mobile phase. This solution was injected into the semi-preparative chiral HPLC system and resolved into each enantiomer on the Chiralcel OD chiral column (250 × 10 mm I.D., Daicel, Tokyo, Japan) by the n-hexane-ethanol-2-propanol-diethylamine (90/5/5/0.25, v/v) as a mobile phase at room temperature and flow rate of 4.0 ml/min monitored at 276 nm UV. Fractions containing single enantiomers were collected and evaporated to dryness under nitrogen stream. Optical purity was determined by the chiral HPLC using Chiralcel OD analytical column (250 × 4.6 mm I.D.) and n-hexane-ethanol-2-propanoldiethylamine (88/6/6/0.25, v/v) as a mobile phase. Optical purity of each enantiomer was 100.0%. (R)-(+)-metoprolol was eluted first (Fig. 1). The retention time of each enantiomer was 6.97 and 11.27 min.

Separation of enantiomers by chiral derivatization method

(–)-Menthyl chloroformate reacted selectively with metoprolol enantiomers to form the corresponding diastereomeric carbamate. The reaction scheme is shown in Fig. 2. The derivatization procedure for metoprolol tartarate was optimized by varing the amounts of the chiral reagent, reaction time and reaction temperature. The optimized conditions are described under materials and methods section. A typical chromatograms of enantiomers of metoprolol after derivatization with (–)-MCF are shown in Fig. 4 and Fig. 5. Under the conditions described in this method, typical values for capacity factors were k_1'=12.14 and k_2'= 13.43 for the (R)-(+)- and (S)-(–)-enantiomers, respectively; the separation factor(α) was 1.11 and resolution (Rs), 1.82. Each of the derivatized enantiomers was separated and collected by semi-preparative reversed-phase HPLC. Then diastereomers were identified by mass spectrometry. The molecular ion peak M^+ of *m/z* 449 was observed for both compounds. Both mass spectra were also identical.

Linearity, recovery and limit of detection

Solutions of each of the enantiomers were spiked with 0.05%~1.00% (w/w) of their stereoisomers. The linearity

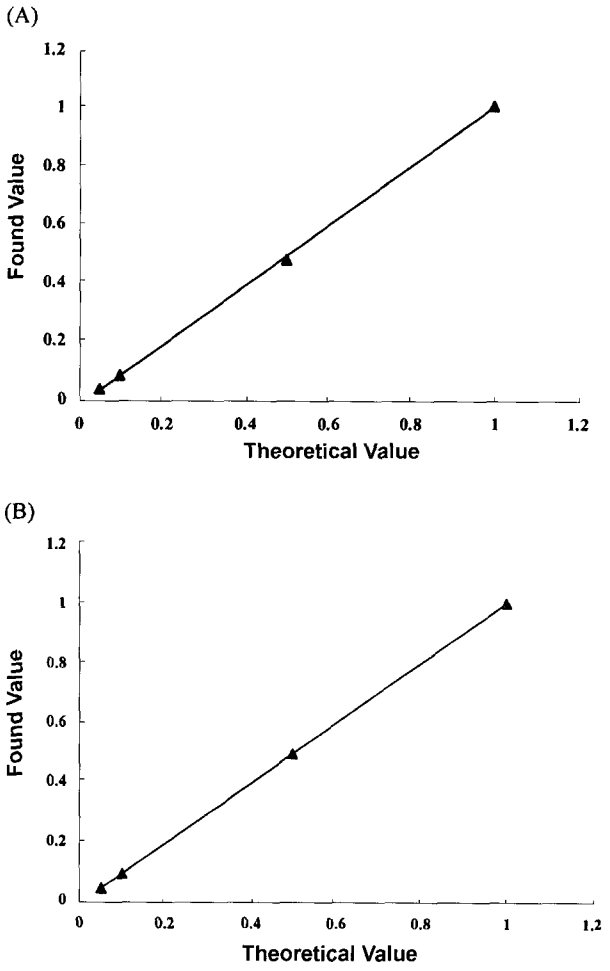

Fig. 3. Linearity of response of (A) (R)-(+)-metoprolol in (S)-(–)-metoprolol and (B) (S)-(-)-metoprolol in (R)-(+)-metoprolol.

and the recovery of peak area response of the (R)-(+)-metoprolol added to (S)-(–)-metoprolol and the (S)-(–)-metoprolol added to (R)-(+)-metoprolol was examined over the range 0.05%~1.00% (w/w) added. The linearity and the recovery testing results are summarized in Table I and Fig. 3, indicating that this method is suitable and applicable for use as the optical purity testing method.

A typical chromatograms of metoprolol enantiomers, spiked with 0.10% of the antipode are shown in Fig. 4 and Fig. 5. In both instances, it was found to be linear over the tested range with a slope of 1.023 and 1.015, an intercept of -0.003 and -0.002 and a correlation coefficient(r) of 0.999 for the (R)-(+)- and (S)-(–)-metoprolol, respectively. The recovery of the antipode was 95.61%~102.59% and the RSD ranged from 0.15% to 3.07%. The detection limit of both enantiomers at a signal to noise ratio of 5 was ca. 0.03%.

Check for racemization

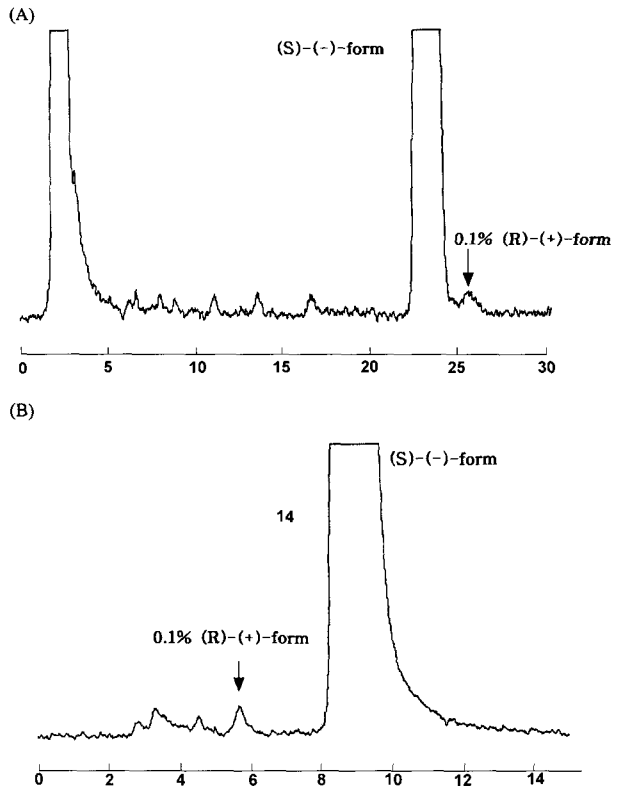

Fig. 4. (A) Reversed-phase chromatogram of the diastereomer obtained from (S)-(–)-metoprolol spiked with ca. 0.1% (R)-(+)-metoprolol after derivatization with (–)-MCF [column, Inertsil C8, 250 × 4.6 mm I.D.; mobile phase, methanol-water (73/27, v/v); flow rate, 1.0 ml/min; detector, fluorescence Ex 276 nm Em 309 nm]. (B) Chiral HPLC chromatogram of (S)-(–)-metoprolol spiked with ca. 0.1% (R)-(+)-metoprolol [column, Chiralcel OD, 250 × 4.6 mm I.D.; mobile phase, n-hexane-ethanol-2-propanol-diethylamine (88/6/6/0.25, v/v); flow rate, 1.0 ml/min; detector, fluorescence Ex 276 nm Em 309 nm].

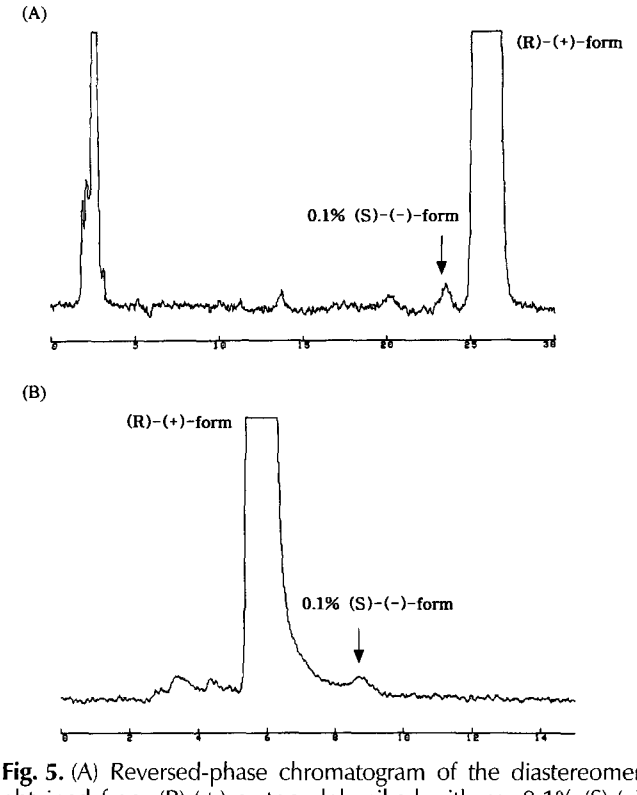

Fig. 5. (A) Reversed-phase chromatogram of the diastereomer obtained from (R)-(+)-metoprolol spiked with ca. 0.1% (S)-(–)-metoprolol after derivatization with (–)-MCF. (B) Chiral HPLC chromatogram of (R)-(+)-metoprolol spiked with ca. 0.1% (S)-(-)-metoprolol. Conditions as in Fig. 4.

To check for racemization during or after the derivatization reaction two methods were compared. The indirect method, derivatization with (–)-menthyl chloroformate, was compared with a direct method, separation on a Chiralcel OD column. The results of the experiments with the mixtures combining different ratios of (R)-(+)- and (S)-(–)-metoprolol are summarized in Table II. The lines for the percentage of (R)-(+)-enantiomer in the sample could be calculated for the two methods according to the equation y=ax+b where y is the percentage of (+) analyzed and × is the percentage of (+) attemped. Direct method : y= 1.0005 × -0.1221 (r=1); Indirect method : y=1.0008 × -0.0087 (r=1); indirect/direct : y= 1.0002 × +0.1147 (r=1). The linearity of the lines with a slope near 1 showed that no racemization occurred during these experiments.

CONCLUSION

It was found that the derivatization of metoprolol with (–)-MCF is an useful technique for the separation of the enantiomers by reversed-phase HPLC. The (–)-MCF

Table I. The linearity and recovery of (R)-(+)-metoprolol and (S)-(-)-metoprolol in the reversed-phase HPLC method after derivatization with (–)-MCF

(R)-(+)-metoprolol				(S)-(–)-metoprolol			
Added (%)	Found (%)	Recovery (%)	C. V. (%)	Added (%)	Found (%)	Recovery (%)	C. V. (%)
0.05	0.051	102.585	3.051	0.05	0.048	95.615	2.549
0.10	0.097	97.085	3.070	0.10	0.098	98.320	0.152
0.50	0.496	99.233	2.689	0.50	0.507	101.310	2.933
1.00	1.025	102.539	2.646	1.00	1.012	101.247	0.866

Table II. Percentages of (R)-(+)- and (S)-(−)-metoprolol obtained by the direct method and the indirect method in samples with various ratios of (R)-(+)- and (S)-(−)-enantiomers

(R)-(+)-metoprolol			(S)-(−)-metoprolol		
Attempted	Analyzed		Attempted	Analyzed	
	Direct	Indirect		Direct	Indirect
100	100.00	100.00	100	100.00	100.00
99	98.99	98.99	99	98.98	98.97
95	95.12	95.06	95	95.18	95.07
70	69.52	70.61	70	70.58	70.52
30	29.42	29.48	30	30.48	29.39
5	4.82	4.93	5	4.88	4.94
1	1.02	1.03	1	1.01	1.01
0	0.00	0.00	0	0.00	0.00

reagent is stable and commercially available. The derivatization procedure is simple and fast. And derivatization reaction of metoprolol with (−)-MCF is independent of the amount of each enantiomer presented in the reaction mixture. The detection limit of the antipode in their stereoisomers was down the 0.03% level. This method could be applied to optical purity testing of drugs containing metoprolol enantiomer.

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