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Chiral Separation of β-Blockers after Derivatization with (-)-α-Methoxy-α-(trifluoromethyl)phenylacetyl Chloride by Gas Chromatography

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Gas chromatographic method was investigated for the chiral separation of several β-blockers(atenolol, betaxolol, bisoprolol, metoprolol and pindolol) using (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as a chiral derivatizing agent for amino group. Prior to N-acylation, hydroxyl group was converted into *O*-silyl ethers by react with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. The reaction was selective and rapid and the diastereomeric derivatives were well separated by capillary gas chromatography. (*R*)-isomers were eluted faster than (*S*)-isomers when (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride was used as the chiral derivatizing agent. But in the opposite sequence when (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride was used. No racemization was found during the reaction.

Key words: Chiral derivatization, β-Blocker, Gas chromatography, Resolution, (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride

INTRODUCTION

β-adrenoceptor blocking agent is used in the treatment of hypertension, angina pectoris and arrhythmias. β-blocking drug sustances most often exhibit a chiral structure and are mostly used as racemic mixtures. 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.

A number of procedures has been reported for the chiral separation of β-blockers, including high-performance liquid chromatography (Peter *et al.*, 2001, Kim *et al.*, 1999, Egginger *et al.*, 1993, Ching *et al.*, 1992, Ekelund *et al.*, 1995, Pirkle *et al.*, 1991, Ceccato *et al.*, 1997, Huynh *et al.*, 1995, Chassaing *et al.*, 1996, Hermansson *et al.*, 1995, Welch *et al.*, 1995 and Srinivas *et al.*, 1992), high-performance capillary electrophoresis (Vargas *et al.*, 1999, Nilsson *et al.*, 1999, Wren, 1995, Siren *et al.*, 1994) and gas chromatography (Gyllenhaal, *et al.*, 1988). Separation of the enantiomers by GC has been achieved either by using an chiral stationary phase or by reacting the enantiomers with a suitable chiral derivatizing reagent. Shin *et al.* (1996) has described the selective *O*-trimethylsilylation-*N*-(-)-α-methoxy-α-(trifluoromethyl) phenylacylation of amphetamines, phenol alkylamines and hydroxyamines and chiral separation of the derivatives by capillary gas chromatography/mass spectrometry with selected-ion monitoring. No publication so far has investigated the selective *O*-trimethylsilylation-*N*-(-)-α-methoxy-α-(trifluoromethyl)phenylacylation of β-blockers.

This paper describes the chiral derivatization of β-blockers with (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and separation of the corresponding diastereomers by capillary gas chromatography.

MATERIALS AND METHODS

Materials and equipment

Atenolol and pindolol hydrochloride were obtained

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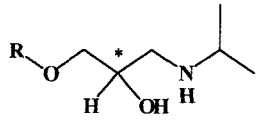
from Il Dong Co. (Seoul, Korea), betaxolol hydrochloride from Bu Kwang Co. (Seoul, Korea), bisoprolol hemifumarate from Yuhan Cyanamide (Kunpo, kyeonggi, Korea), and metoprolol tartrate from Yuhan Co. (Seoul, Korea). *N*-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride((-)-MTPA-Cl) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Other reagents as a analytical grade were obtained from Duksan Pure Chemicals Co.(Ansan, Kyeonggi, Korea).

The chiral semi-preparative high performance liquid chromatographic system consisted of LC-9A pump, C-R4A intergrator, SPD-6AV detector(Shimadzu, Kyoto, Japan) with a semi-preparative flow cell and Rheodyne 7725*i* injector with a 200 μl loop. The chiral analytical high performance liquid chromatographic system consisted of LC-10A pump, SIL-10A autoinjector, CLASS-10LC with CBM-10A computerized intergrator (Shimadzu, Kyoto, Japan), and 502T UV detector (GL-Science, Tokyo, Japan) with a analytical flow cell. A gas chromatograph (Carlo-Erba 8000) equipped with a capillary column BP-5 (cross-linked 5% phenymethylsilicone; 25 m × 0.22 mm × 0.25 m film thickness) was used for the analysis of derivatives of β-blockers. The flow rate of carrier gas (He) was 1.0 ml/min. The split ratio was 10:1. Both the injector and detector (FID) were set at 300°C. The oven temperature gradient was used. Temperature was increased from 100 of initial value to 310°C by 3°C per minute with holding 10 min at final temperature. Autospec M393 Mass Spectrometer was coupled with Carlo-Erba 8000 GC. Ionization potential was 70 eV. Transfer line temperature was 300°C.



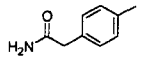
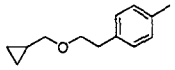
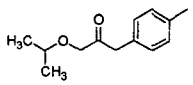
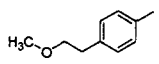
β - Blockers	R
Atenolol	
Bisoprolol	
Betaxolol	
Metoprolol	
Pindolol	

Fig. 1. Structures of β-blockers. Chiral center is indicated by an asterisk.

Preparation of the enantiomers of β-blockers

100 mg of each β-blocker was dissolved in 10 ml of mobile phase (n-hexane-isopropanol-diethylamine). This solution was injected into the chiral semi-preparative HPLC system and resolved into each enantiomer on the Chiralcel OD chiral column (5 μm, 250 × 10 mm I.D., Daicel, Japan) at room temperature at a flow rate of 4.0 ml/min monitored at UV 276 nm. 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 (5 μm, 250 × 4.6 mm I.D., Daicel, Japan).

Derivatization procedures

Stock solutions of the various β-blockers (1 mg/ml) were prepared in acetonitrile. Aliquots of these solutions (50 μl) were pipetted into a screw-capped tube, evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 50 μl of a mixture of acetonitrile-trifluoroacetic acid (60:40, v/v) that contained 200 μg/ml of methyl orange. The mixture was titrated with MSTFA until the color of the reaction mixture changed from red to yellow. The sample was heated for 5 min at 60°C on a heat block. Then 5 μl of (-)-MTPA-Cl was added to the reaction mixture and the the sample was heated for an additional 5 min at 60°C (Fig. 2).

RESULTS AND DISCUSSION

Preparation of the enantiomers of β-blockers

β-Blocker racemate was separated to each enantiomer

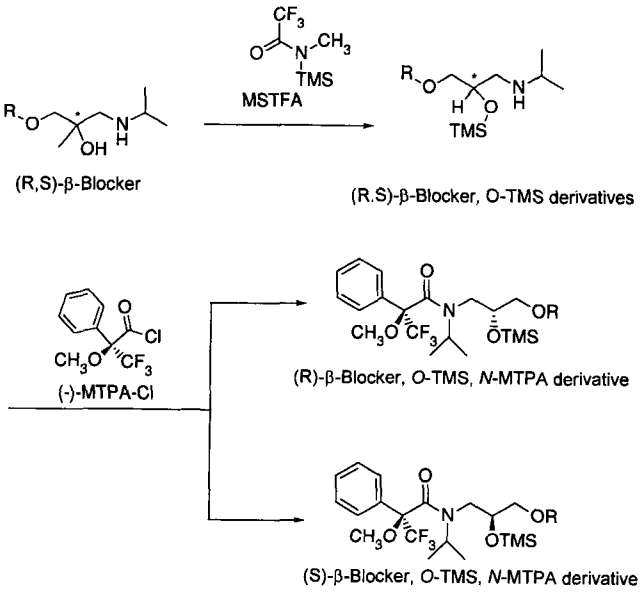


Fig. 2. Derivatization reaction of β-blockers using MSTFA and (-)-MTPA-Cl

by chiral semi-preparative HPLC within 30 min. All the (*R*)-enantiomers of β-blockers used in this study eluted faster than (*S*)-enantiomers. White-colored amorphous powder of each enantiomer was obtained. Enantiomeric purity was 100.0% for each enantiomer. These enantiomers could be used as standards for elution order of *N*-(-)-MTPA-*O*-silylated diastereomers of β-blockers and chiral conversion test of β-blockers during the derivatization reaction.

Derivatization

β-Blockers used in this study contain amino and hydroxyl group suitable for derivatization. The hydroxyl group reacts readily with silylating reagent (MSTFA) under mild conditions. MTPA-Cl has been shown to be a useful chiral reagent for GC. β-Blockers were examined after conversion to the diastereomeric amides, prepared respectively by selective *O*-silylation with MSTFA, followed by selective *N*-acylation with (-)-MTPA-Cl. By titrating with silylating agent until the color change of the reaction mixture, use of excess silylating agent was avoided.

The relationship between the yield of *N*-MTPA, *O*-TMS derivative of β-blockers and the reaction time was evaluated. Maximum yield of the derivative was determined immediately after addition of reagent and no trace amount of underivatized substance was detected in the reaction mixture after addition of reagents. The reactions of the enantiomers proceeded at the same rate. An

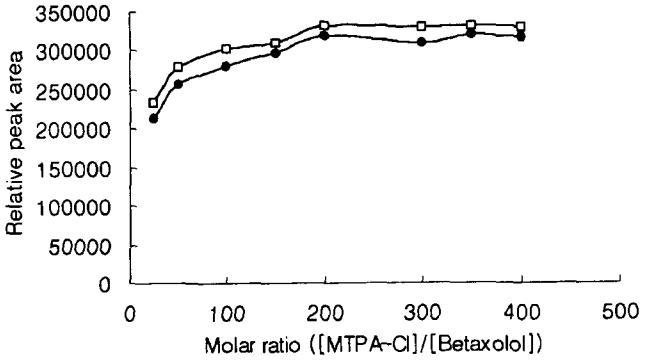


Fig. 3. Effect of the concentration of (-)-MTPA-Cl on the derivatization of betaxolol.

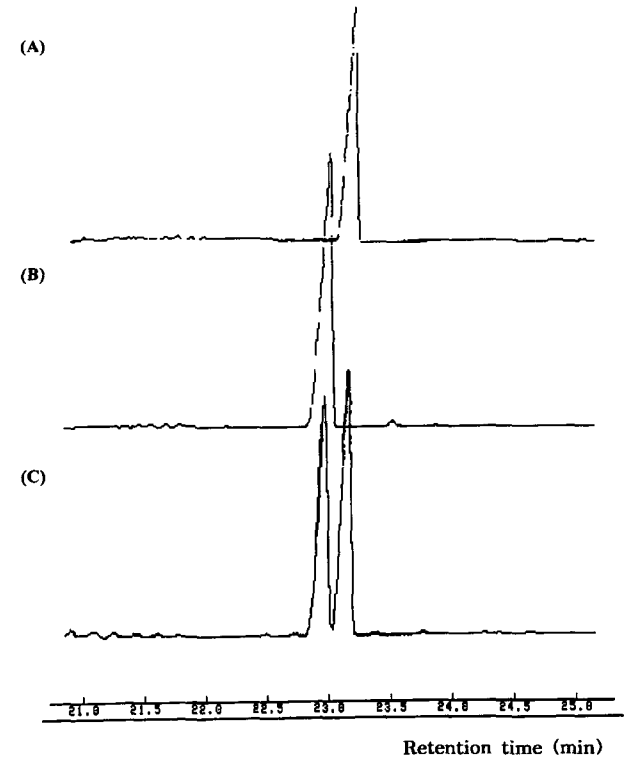


Fig. 4. GC chromatogram of (A) *S*-(-)-metoprolol, (B) R-(+)-metoprolol, (C) metoprolol racemate.

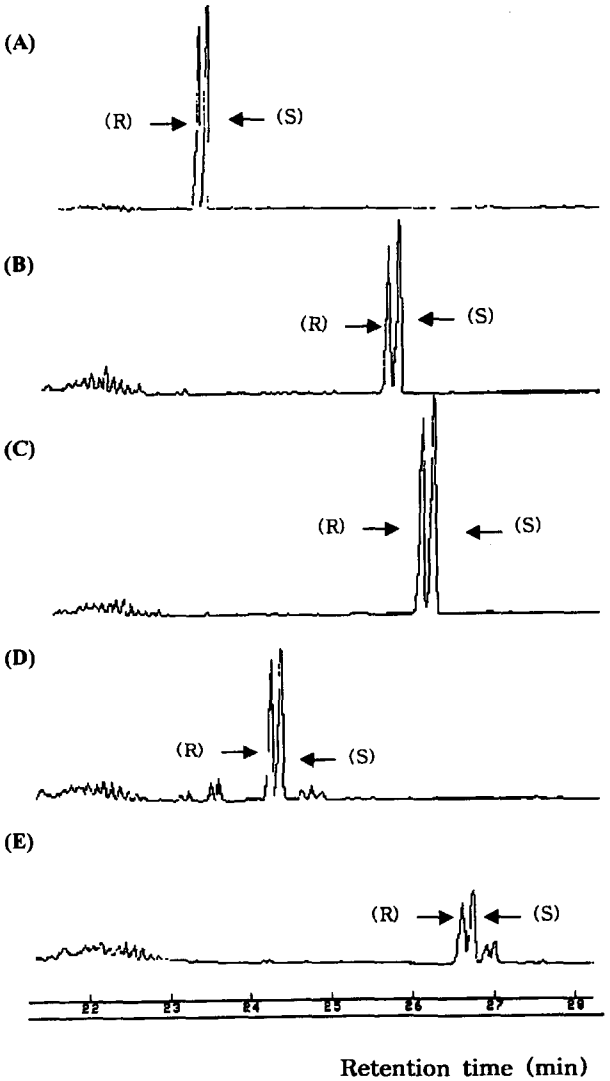


Fig. 5. GC chromatogram of *N*-MTPA, *O*-TMS, derivatives of β-blockers. (A) atenolol, (B) betaxolol, (C) bisoprolol, (D) metoprolol, (E) pindolol.

Table I. Characteristics of the mass spectra of *N*-MTPA, *O*-TMS derivatives of β-blockers

N-MTPA, *O*-TMS derivatives of β-blockers	MW	Characteristic ions (m/z)
Atenolol	554	189(100), 347(47.4), 404(35.8), 73(35.1), 105(21.3), 282(20.5), 119(11.1), 521(9.3), 390(6.4), 554(0.01).
Betaxolol	594	404(100), 189(51.6), 405(27.1), 73(22.5), 281(10.9), 105(8.2), 282(6.3), 580(5.5), 594(0.03).
Bisoprolol	613	404(100), 189(62.4), 73(31.8), 105(10.4), 282(9.3), 419(8.6), 221(7.2), 390(5.5), 613(0.1).
Metoprolol	555	404(100), 189(66.4), 73(25.6), 366(10.4), 105(10.0), 235(7.3), 159(5.6), 555(0.1)
Pindolol	536	404(100), 189(39.1), 73(15.6), 133(11.2), 105(9.0), 363(5.4), 158(5.2), 521(3.2), 536(1.3).

Table II. Chromatographic results of *N*-MTPA, *O*-TMS derivatives of β-blockers on the gas chromatography

β-blockers	Chromatographic parameters				
	k'_R	k'_S	N_R	N_S	Resolution (R_S)
Atenolol	18.07	18.18	1016871	1032256	1.44
Betaxolol	19.83	20.00	581864	589210	1.18
Bisoprolol	19.54	19.66	748571	799236	1.30
Metoprolol	17.37	17.46	764576	772289	1.17
Pindolol	19.24	19.36	726586	686247	131

k'_R : Capacity factor of *N*-MTPA, *O*-TMS derivatives of (*R*)-enantiomer, k'_S : Capacity factor of *N*-MTPA, *O*-TMS derivatives of (S)-enantiomer, N_R: The number of theoretical plates of N-MTPA, *O*-TMS derivatives of (*R*)-enantiomer, N_S: The number of theoretical plates of *N*-MTPA, *O*-TMS derivatives of (*S*)-enantiomer.

increase of the concentration of (-)-MTPA-Cl led to a general increase in formation of the diastereomers upto 200 times molar excess of (-)-MTPA-Cl and reached a plateau (Fig. 3).

To check for racemization during or after derivatization reaction, enantiomeric pure (*R*)-(+)-metoprolol and (*S*)-(-)-metoprolol were derivatized as described above. No racemization was found (Fig. 4).

Mass spectrometry

Fragment ions of derivatives of β-blockers were obtained by electron impact ionization at 70 eV. Molecular ions were of low abundance under the given experimental condition. *N*-MTPA derivative afforded the characteristic ion at m/z 189, due to cleavage adjacent to the carbonyl group from the derivative reagent. *N*-MTPA, *O*-TMS derivatives of β-blockers, except atenolol, gave the base ion peak at m/z 404, due to the cleavage adjacent to the oxygen atom from the skeleton of β-blockers (Table I).

Chromatographic behaviour of the derivatives

N-MTPA, *O*-TMS derivatives of β-blockers were well separable by capillary GC (Fig. 5). When (-)-MTPA-Cl was used, *N*-MTPA, *O*-TMS derivatives of (*R*)-enantiomers of all the β-blockers used in this study were eluted faster than those of (*S*)-enantiomers but in the opposite sequence when (+)-MTPA-Cl was used. A detailed presentation, giving capacity factors (k'), resolution (R_S) and the number of theoretical plates (N) of the chromatographic results obtained by the above mentioned procedures is shown in Table II. The peak resolution values(Rs), ranging between 1.17 to 1.44, were obtained.

CONCLUSION

Stereoselective method for the separation of enantiomers of β-blockers has been developed by gas chromatography. The hydroxyl and amino group of β-blockers were reacted readily with MSTFA and (-)-MTPA-Cl under mild conditions. *N*-MTPA, *O*-TMS derivatives of β-blockers were suitable for identification and quantitation by gas chromatography. No racemization was found during the experiment. This method could be applicable to the chiral separation of β-blockers in biological fluids.

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