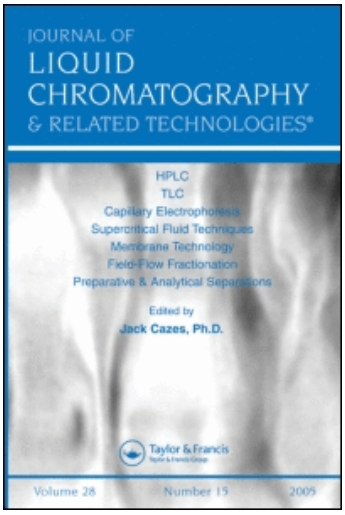

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CHIRAL SEPARATION OF ACEBUTOLOL BY DERIVATIZATION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY#

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ABSTRACT

A simple and specific high performance liquid chromatographic (HPLC) method is described for the determination of R-(+)- and S-(-)acebutolols.

The method is based on the derivatization of the hydroxyl and amino functions of acebutolol with N-benzyloxycarbonyl-L-phenylalanine and acetic anhydride, respectively. The resulting derivatives were separated on a reversed phase C-18 column and monitored with a UV detector, leading to a base line resolution of the diastereomeric derivatives of S-(-) and R-(+) acebutolols.

#This work was presented in part at the annual meeting of the Pharmaceutical Society of the R.O.C., Taipei, Dec. 17th, 1994.

INTRODUCTION

It has been reported [1-4] that enantiomers of certain β–adrenergic blockers have quite different biological activities and pharmacokinetic profiles. Therefore, the stereospecific analysis of β–adrenergic blockers has recently received widespread attention [2,5-6].

Acebutolol is one of β–adrenergic blockers that are frequently used for the treatment of the diseases related to hypertension and ventricular arrhythmia [7]. Acebutolol is a chiral compound but its racemic form is used as a drug for clinical treatment. Several chiral reagents including (S)-(-)-N-trifluoroacetyl-L-prolyl chloride (TPC) [8], (R)-(+)-1-phenyl-ethyl isocyanate [9], (S)-(-)-1-phenylethyl isocyanate [6] and (S)-(+)-1-(1-naphthyl) ethyl isocyanate [10] have been used for the enantiomeric analysis of acebutolol as diastereomers, but TPC is unstable and easily racemized in storage [8]. The isocyanate reagents are moisture sensitive and must be handled with care.

In this study, a modification method based on our previous report [11] using commercially available N-benzyloxycarbonyl-L-phenylalanine (N-CBZ-L-Phe) and acetic anhydride was developed for the chiral separation of racemic acebutolols. Derivatization of acebutolol with N-CBZ-

L-Phe and acetic anhydride was conducted in mild and simple conditions. The resulting derivatives from racemic acebutolol can be easily separated as the diastereomers on a reversed phase C-18 column and monitored with a UV detector.

METHODS

Chemicals and Solutions

N-Acetyl-O-(N-benzyloxycarbonyl)-L-phenylalanyl acebutolol (ACB-D) was synthesized in our laboratory, and its structure was confirmed by MS, NMR and elemental analysis. Acebutolol was simply prepared by neutralization of acebutolol hydrochloride (May & Baker, Dagenham, U.k.) with sodium bicarbonate solution and structurally identified by MS, NMR and elemental analysis. 9-Acetylanthracene (Aldrich, Milwaukee, WI, U.S.A.), N-benzyloxycarbonyl-L-phenylalanine (N-CBZ-L-Phe) (Sigma, St. Louis, MO, U.S.A.), 4-N, N-dimethylaminopyrine (DMAP) (Tokyo Kasei, Tokyo, Japan), acetic anhydride (Ac_2O), toluene, dicyclohexylcarbodiimide (DCC) and silica gel 60 for column chromatography (Merck, Darmstadt, Germany), dichloromethane (without ethanol as stabilizer), methanol, acetonitrile and tetrahydrofuran

(THF) of HPLC grade (Fisher, Springfield, NJ, U.S.A.), ethyl acetate, n-hexane and acetone (Lab-Scan, Dublin, Ireland) were used without further purificiation. All other chemicals were of analytical reagent grade.

Solutions of 9-acetylanthrancene (internal standard, I.S.), acebutolol, DMAP, DCC, N-CBZ-L-Phe and acetic anhydride at various concentrations were prepared by dissolving the respective compound in dichloromethane. Deionized and distilled water was used to prepare related aqueous solutions.

Liquid Chromatography

Isocratic HPLC conditions consisting of a Waters Millipore 501 LC pump, a U6K injector, a C-18 reversed phase column (Waters Millipore, 150 X 3.9 mm I.D., 4 μm) with a disposable Nova-Pak C-18 precolumn (10 μm; bed volume < 100 μL) and a Waters 486 tunable absorbance detector were applied. The mobile phase of methanol/water (60/40, v/v), degassed with vacuum filter through a 0.45-μm filter, was used at a flow rate of 1.3 mL/min and the column eluate was monitored at 254 nm.

Mass Spectrometry

Mass spectrum was obtained on a VG Biotech Quattro 5022 mass spectrometer with fast atom bombardment (FAB).

Derivatization Procedure

A 0.5-mL volume of acebutolol solution was added to a 10-mL glass-stoppered test tube pre-cooled in an ice bath, containing 0.1 mL of N-CBZ-L-Phe solution (55 mM), 0.1 mL of DMAP solution (14 mM) and 0.1 mL of the I.S. solution (95 nM). Then 0.1 mL of DCC solution (0.24 M) was added and the reaction mixture was shaken mechanically at 0 ° C for 0.5 h shaker. At the end of the reaction, 0.1 mL of acetic anhydride (1.06 M) was added and the resulting reaction mixture was shaken mechanically at 30 ° C for 15 minutes in a thermostated shaker. After the reaction, 1.0 mL of methanol was added and mixed. The resulting solution was used for HPLC analysis.

RESULTS AND DISCUSSION

In order to optimize the derivatization conditions for acebutolol, several parameters such as reaction solvent, the concentrations of DMAP and N-CBZ-L-Phe, the amounts of DCC and acetic anhydride, and reaction time were investigated. The amount of acebutolol used for study was 0.34 μmol (in 0.5 mL of dichloromethane solution), unless otherwise indicated. The effects of the parameters on the derivatization

of acebutolol were evaluated by the peak-area ratios of the derivative to the I.S.

Effect of Reaction Solvent

The effects of various organic solvent (excluding alcohols) on the derivatization of acebutolol were studied according to the Derivatization Procedure. The solvents tested included acetone, acetonitrile, dichloromethane, ethyl acetate, THF and toluene. Dichloromethane was found to be the best solvent for the derivatization.

Effect of N-CBZ-L-Phe

The concentration of N-CBZ-L-Phe solution (0.1 mL) required for the derivatization of acebutolol to a plateau formation of the derivative is around 36 mM as shown in Figure 1, but a higher concentration of N-CBZ-L-Phe solution at 55 mM was selected for the derivatization, equivalent to a molar ratio (N-CBZ-L-Phe to acebutolol) of 16.6.

Effect of DMAP

The effect of the DMAP solution (0.1 mL) at various concentrations on the formation of the acebutolol derivative is shown in Figure 2. The results indicate that the concentration range of DMAP is suitable

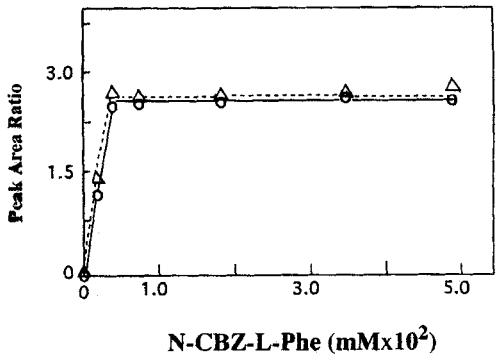

FIGURE 1. Effect of N-CBZ-L-Phe concentration on the formation of acebutolol derivatives. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

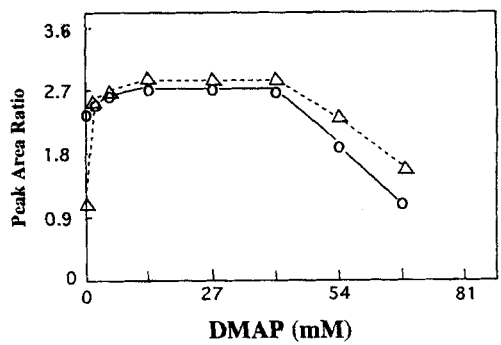

FIGURE 2. Effect of DMAP concentration on the formation of acebutolol derivatives. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

over 6.85- 40.5 mM, and DMAP solution at the concentration of 14 mM was used for derivatization.

Effect of DCC

The effect of DCC solution (0.1 mL) at varied concentrations up to 0.5 M on the formation of the acebutolol derivative was studied. As shown in Figure 3, the optimal concentration of DCC for the derivatization of acebutolol is above 0.087 M and DCC at 0.24 M was used for derivatization.

Effect of Acetic Anhydride

The concentration of Ac_2O solution (0.1 mL) required for the derivatization of acebutolol to a plateau formation of the derivative is around 0.04 M as shown in Figure 4, but a higher concentration of this simple reagent at 1.06 M was used for the derivatization.

Effect of Reaction Time

The reaction time required for the esterification of acebutolol with N-CBZ-L-Phe to give a constant formation of the acebutolol ester is about 10 minutes at 0 ° C as shown in Figure 5; and that for the acetylation of the acebutolol ester to give a constant formation of the final

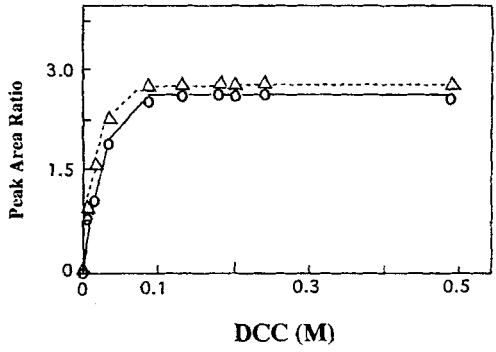

FIGURE 3. Effect of DCC concentration on the formation of acebutolol derivatives. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

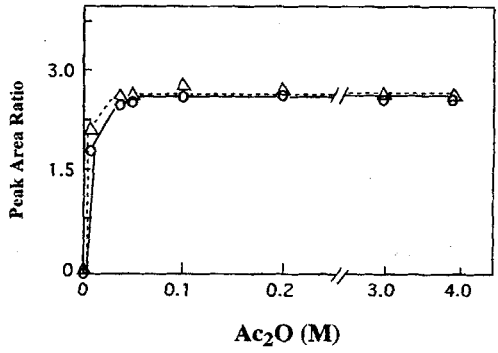

FIGURE 4. Effect of Ac_2O concentration on the formation of acebutolol derivatives. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

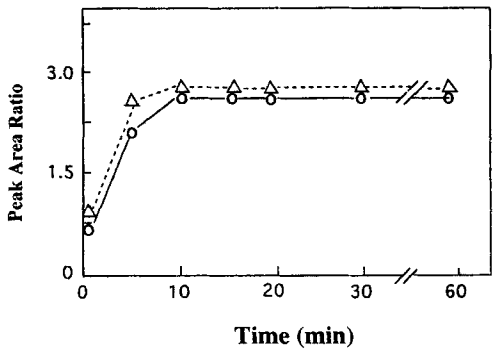

FIGURE 5. Effect of reaction time on esterification of acebutolols. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

derivative is about 10 minutes at 30 ° C as shown in Figure 6. The reaction times for esterification and acetylation were set 30 min and 15 min, respectively.

Based on the optimum derivatization conditions obtained above, a derivatization procedure was formulated under the METHODS section. Derivatization of acebutolol only with N-CBZ-L-Phe resulted in an ester derivative which was unstable, reflecting on gradually decreasing the peak-area ratios of the derivative to the I.S. after derivatization, due probably to the intramolecular degradation as postulated in Figure 7. Therefore, derivatization of acebutolol successively with esterification and acetylation is essential. The resulting derivative is stable up to 4 days tested as shown in Figure 8.

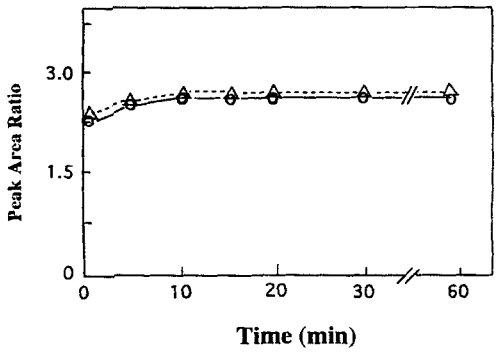

FIGURE 6. Effect of reaction time on acetylation of acebutolol esters. o : S-acebutolol derivative; Δ : R-acebutolol derivative.

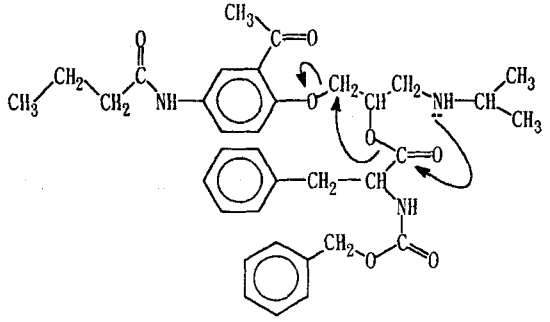

FIGURE 7. Assumed intramolecular degradation of O-(N-benzyloxycarbonyl)-L-phenylalanyl acebutolol.

Analytical Calibration and Typical Chromatograms

To evaluate the quantitative applicability of the method, six different concentrations of acebutolol over the range 0.01- 0.32 μmol, (each in 0.5 mL of dichloromethane solution) were determined to construct a calibration graph. The results indicate good linearity for the determina-

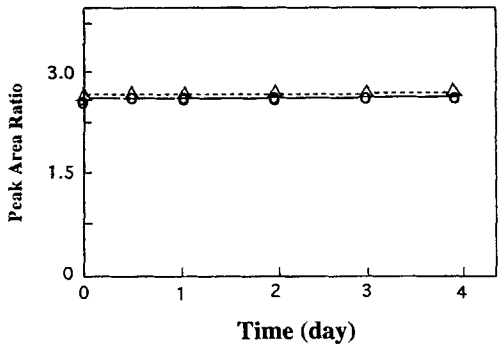

FIGURE 8. Stability of acebutolol derivatives after derivatization.
o : S-acebutolol derivative; Δ : R-acebutolol derivative.

tion of acebutolol over the range studied; linear regression equations, $y = 9.5497\chi - 0.0031$ (for S-acebutolol) and $y = 9.7451\chi - 0.0016$ (for R-acebutolol) were obtained with correlation coefficients of 0.9997 and 0.9998, respectively; where y is the peak-area ratios of the acebutolol derivative to the I.S., and χ is the amount of acebutolol in μmol. The derivatization yield of acebutolol is nearly quantitative (> 94%) as presented in Table 1, based on the calculation of the peak-area ratios of acebutolol tested to the I.S. in comparison with that of the acebutolol derivatives (ACB-D) synthesized. The observed C.V. for the intra-assay (n=6) at two levels of acebutolol is below 5% as shown in Table 2.

A typical liquid chromatogram is presented in Figure 9, illustrating a good resolution of the acebutolol derivatives (Resolution >1.5) and they

TABLE 1

Derivatization Yield of Acebutolol

Acebutolol tested(μmol)	The derivative found(μmol)*	Yield(%)
S 0.16	0.15 ± 0.009	94
R 0.16	0.16 ± 0.006	102
S 0.32	0.32 ± 0.004	99
R 0.32	0.34 ± 0.004	106
S 0.64	0.65 ± 0.011	102
R 0.64	0.69 ± 0.013	108

* Mean ± S. D. of three replicate analyses.

TABLE 2

Precision for the Determination of Acebutolol

Acebutolol tested(μmol)	The derivative found(μmol)*	C.V. %
S 0.06	0.06 ± 0.003	4.8
R 0.06	0.06 ± 0.001	2.1
S 0.44	0.45 ± 0.004	1.0
R 0.44	0.44 ± 0.008	1.8

* Mean ± S. D. of three replicate analyses.

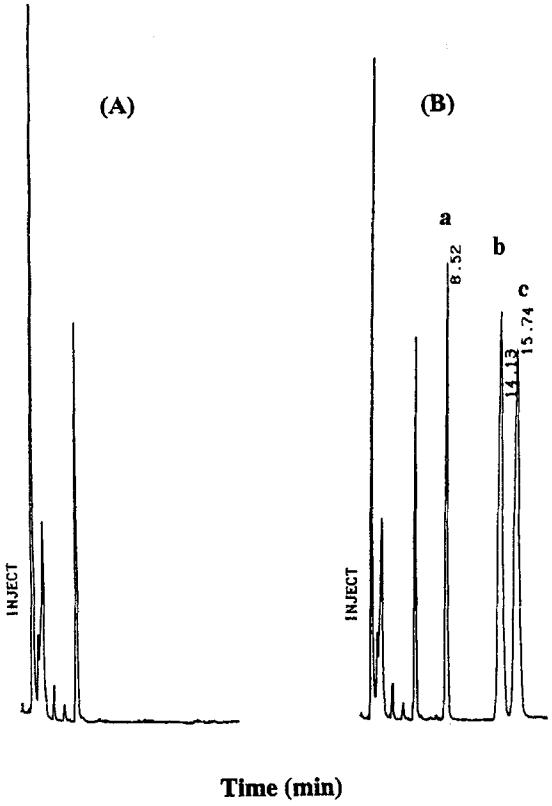

FIGURE 9. HPLC chromatograms of (A) reagent blank (B)derivatives of enantiomeric acebutolol. Peaks: a, internal standard; b, S-acebutolol derivative; c, R-acebutolol derivative. HPLC conditions: column, Nova-Pak C18(15cm x 3.9 mm I.D.); mobile phase, 60%(v/v) methanol in water; flow rate 1.3 mL/min; detector, UV 254 nm, 0.02AUFS.

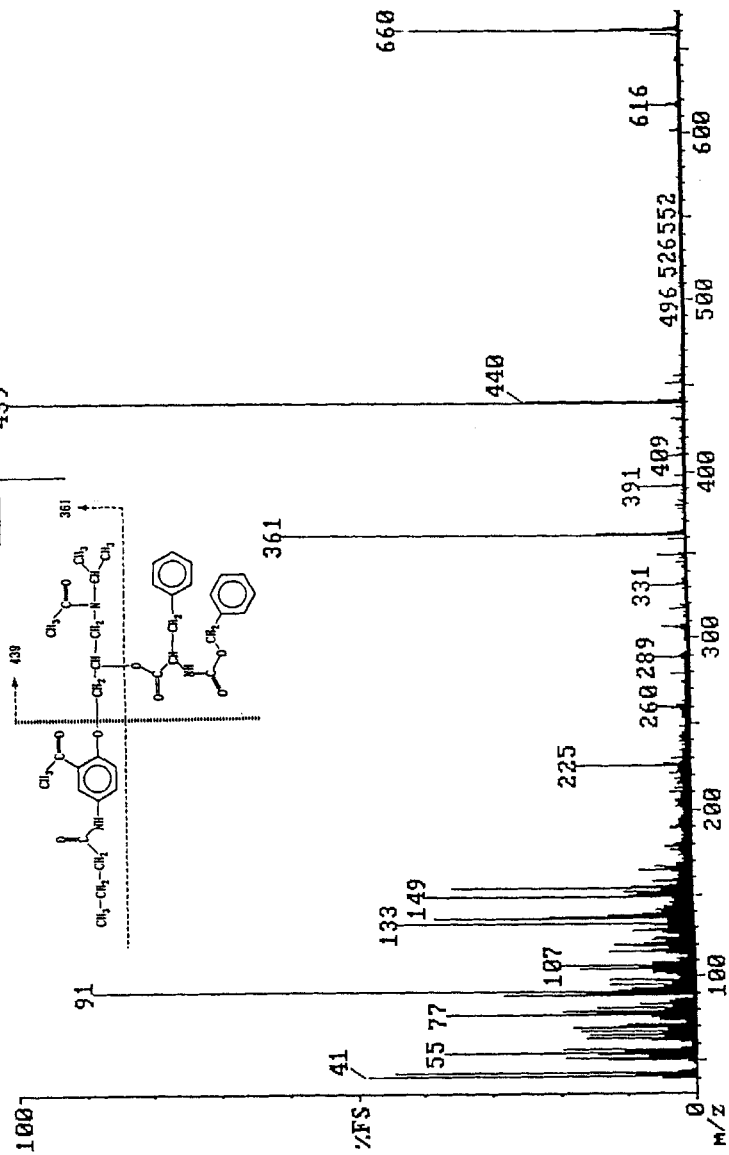

FIGURE 10. FAB-MS spectrum of acebutolol derivative. See text for conditions.

are eluated in reasonable time (16 min). The structure of the acebutolol derivative isolated was determined by FAB-MS (Figure 10) with nitrobenzyl alcohol as a matrix, giving a pseudomolecular ion at m/z=660 (M +H) and a diagnostic peak at m/z=361, equivalent to the fragment of a substituted acetamide (CH_3CONRR'). This indicates that the amino nitrogen of acebutolol is acetylated. Therefore, the derivative is tentatively assigned as N-acetyl-O-(N-benzyloxycarbonyl)-L-phenylalanyl acebutolol (ACB-D).

In conclusion, a simple and specific HPLC method was developed for the chiral analysis of acebutolol, based on the derivatization of acebutolol with N-CBZ-L-Phe and acetic anhydride in mild conditions. Further application of the method to the chiral analysis of related β-blocker alcohols will be very attractive.

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REFERENCES

1. G. Pflugmann, H. Spahn and E. Mutschler, J. Chromatogr., 421: 161-164, (1987).

2. L.S. Olanoff, T. Walle, K. Walle, T.D. Cowart and T.E. Gaffney, Clin. Pharmacol. Ther., 35: 755-761, (1984).
3. U.K. Walle, T. Walle, S.A. Bai and L.S. Olanoff, Clin. Pharmacol. Ther., 34: 718-723, (1983).
4. C. Von Bahr, J. Hermansson and K. Tawara, Br. J. Clin. Pharmacol., 14: 79-82, (1982).
5. M.J. Wilson, K.D. Ballard and T. Walle, J. Chromatogr., 431: 222-227, (1988).
6. P.H. Hsyu and K.M. Giacomini, J. Pharm. Sci., 75: 601-605, (1986).
7. G. De Bono, C.M. Kaye, E. Roland and A.J.H. Summers, Am. Heart J., 109: 1211-1213, (1985).
8. M.G. Sankey, A. Gulaid and C.M. Kaye, J. Pharm. Pharmacol., 36: 276-277, (1983).
9. A.A. Gulaid, G. W. Houghton and A.R. Boobis, J. Chromatogr., 318: 393-397, (1985).
10. M. Piquette-Miller, R.T. Foster, F.M. Pasutto and F. Jamali, J. Chromatogr., 526: 129-137, (1990).
11. S.S. Wu, Y.H. Wen, Y.F. Wang and H.L. Wu, J. Liq. Chromatogr., 12: 2997-3005, (1989).

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