Southeast-Asian J. of Sciences Vol. 4, No. 2 (2016) pp. 93-101

PREPARATION OF FOUR STEREOISOMERS OF LABETALOL USING S-(-)-ALPHA-METHYLBENZYL ISOCYANATE AS A CHIRAL DERIVATIZING REAGENT

Nguyen Thi Ngoc Van[1], **Seung-Ho Choi**[2]
and **Kyeong Ho Kim**[2]

[1] *Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy*
179 Nguyen Van Cu street, Ninh Kieu District, Can Tho city, Viet Nam
e-mail: ntnvan@ctump.edu.vn

[2] *College of Pharmacy, Kangwon National University*
Chuncheon 200-701, Republic of Korea
e-mail: kyeong@kangwon.ac.kr

Abstract

Stereoisomers of labetalol were derivatized with (S)-(-)-alpha- methylbenzyl isocyanate (S-(-)-MBIC) forming their diastereomers, R,S- labetalol-S-(-)-MBIC, S,R-labetalol-S-(-)-MBIC, R,R-labetalol-S-(-)-MBIC, and S,S-labetalol-S-(-)-MBIC. Diastereomeric mixture was then chromatographically resolved by semi-preparative HPLC (Inertsil ODS C_{18} column, 250 mm x 10 mm i.d. 5 m) eluted with methanol- water (56: 44, v/v) at a flow rate of 3 mL/min. Then, the diastereomers were hydrolyzed with 30 mM H_2SO_4 to produce their stereoisomers at 60^oC for 56 hours, and the decomposed mixture was further purified by semi-preparative HPLC. The stereoisomeric purity and final yield of four stereoisomer of labetalol were at least 99.95% and 16%, respectively.

1. Introduction

The necessity to generate individual enantiomers for testing is a growing priority in pharmaceutical research and discovery. Numerous methods can be

Key words: (S)-(-)-alpha-methylbenzyl isocyanate, Labetalol, Stereoisomers, Diastereomer, Chiral derivatizing reagent.

employed to obtain enantiopure compounds. One approach is the asymmetric synthesis of the desired enantiomer, which requires two synthetic routes to be developed and usually uses "chiral pool", chiral auxiliaries or enantioselective catalysis to achieve the desired goals. Another approach is the resolution of an enantiomeric mixture that has the advantage of producing both enantiomers. In the latter, this can be achieved by diastereoselective salt formation, conglomerate, kinetic or chromatographic resolution [1]. Liquid chromatography is now the most accepted method for chiral separations, not only in the direct way, using chiral stationary phases, but also in the indirect way, by using chiral derivatizing reagents [2, 3].

Labetalol, (RS)-2-hydroxy-5-{1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl} benzamide was the first antihypertensive drug with both α - and β-adrenoreceptor blocking properties [4]. It was a β-blocker drug widely used in the management of hypertension and angina pectoris [5]. The compounds possess two chiral centers and can exist in four stereoisomeric forms (R,R), (S,S), (R,S), and (S,R). The (S,R)-isomer, is a powerful α_1- blocker. The (RR)-isomer exhibits mainly a β_1-antagonist activity with some α_1 -antagonism and β_2 agonist activity [6]. The (S,S) and (R,S) isomers appear to be pharmacologically inactive. In 1990, a single isomer (R,R) marketed but was subsequently withdrawn due to hepatotoxicity [7]. A gender difference in labetalol kinetics research was reported that concentrations of three of the four stereoisomers of labetalol were higher in women than men [8]. This study highlights the importance of determining stereoisomers kinetics for drugs administered as racemates. Another pharmacokinetic analysis of labetalol stereoisomers has been applied to hypertensive pregnant women [9]. Such evidence demonstrates that kinetic, pharmacological, and toxicological properties of individual stereoisomers of labetalol need to be carefully characterized in further studies. Labetalol is currently being marketed as a racemic mixture of all four stereoisomers. Hence, it is particularly importance in the pharmaceutical industry to obtain optically pure stereoisomer of labetalol to study potential stereoselective differences in the metabolic disposition of labetalol stereoisomers and/or metabolites.

The aim of this work was stereoisomeric preparation of each stereoisomer of labetalol using a chiral derivatization method. In this paper, chiral derivatization following chromatographic resolution was combined with hydrolysis to prepare optically pure stereoisomers. Labetelol was derivatized with S-(-)-MBIC) forming its diastereomers, R,S-labetalol-S-(-)-MBIC, S,R-labetalol-S-(-)-MBIC, R,R-labetalol-S-(-)-MBIC, and S,S-labetalol-S-(-)-MBIC. Then the diastereomers were hydrolyzed with 30 mM H_2SO_4 to produce stereoisomer and purified by reverse phase HPLC, quantitatively.

2. Materials and methods

Materials Labetalol obtained from Sigma-Aldrich (Milwaukee, WI, USA). (S)-(-)- -methylbenzyl isocyanate (S-(-)-MBIC) was purchased from TCI Fine Chemicals (Tokyo, Japan). Diethyl amine as an analytical grade was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Ethanol, n-hexane, methanol as an HPLC grade was obtained from Duksan Pure Chemicals Co. (Ansan, Korea). Sulfuric acid as acidic catalyst was from Junsei (Tokyo, Japan). All other reagents as an analytical grade obtained from DaeJung Chemicals and Materials Co. (Siheung, Korea).

Apparatus

The chiral HPLC equipment consisted of LC-10AD pump, RF-10AXL Fluorescence detector (Shimadzu), monitored at an excitation wavelength of 330 nm and with an emission wavelength of 418 nm, with Chirobiotic- V column (4.6 mm x 150 mm i.d. 5μm) and 20 μl-injection loop. The achiral HPLC equipment consisted of PU-980 pump, UV-975 detector (Jasco) and 20 μl-injection loop with phenomenex ODS column (4.6 mm 250 mm i.d. 5 m), detection wavelength was set at 210 nm. The semi-preparative HPLC equipment consisted of LC-20AD pump (Shimadzu, Japan), Lambda-Max Model 481 detector (Waters, USA) with Inertsil ODS C18 column (10 mm x 250 mm i.d. 5μm, GL Science).

NMR spectroscopy was performed with Brucker Advance-600 (600.13 MHz, Brucker, Germany) operating at probe temperature of 23 $\pm 1^o$C and was reference to TMS taken as 0.00 ppm on the δ scale. For mixing the contents of NMR tube, Maxi Mix II (Barnstead/ Thermolyne, USA) mixer was used. Spectra were recorded at 600 MHz with CDCl3 99.8 atom% D contains 0.05% (v/v) TMS as an internal standard.

Derivatization of labetalol with a chiral derivatizing reagent Stock solution of labetalol (0.33 mg/mL) was prepared in 50% of acetonitrile in water, 1 mL were placed into distinct 5 mL vial. To this, 1 mL of 43 mM S-(-)-MBIC solution added, and those were mixed together by vortex in 10 seconds. Mixture of two compounds was stirred at room temperature, 40, 50, 60, 70oC in the range 30 - 90 min. The effect of reaction time and temperature were investigated through total peak area of diastereomeric derivatives.

The effect of S-(-)-MBIC concentration on the derivatization investigated in the range from 2- 12 mM. The sample concentration was fixed at 1 mM. The derivatives produced were injected on to the HPLC after a ten fold dilution and were detected at 210 nm. The derivatization reaction is depicted in Fig. 1. All handling with labetalol were done in subdued light to prevent decomposition.

Fractionation of four labetalol-MBIC diastereomers by semi- preparative HPLC

From the labetalol-S-(-)-MBIC diastereomerics mixture, each diastereomer

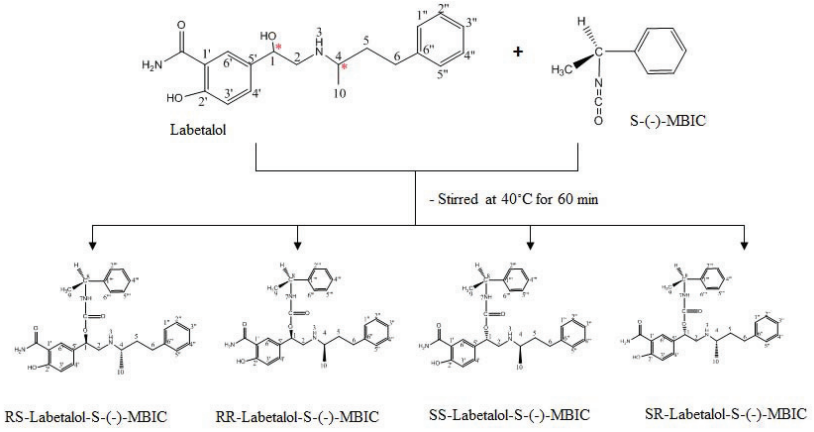

Fig. 1. Reaction between labetalol and S-(-)-MBIC leading to the formation of four labetalol derivatives

was fractionated by semi-preparative HPLC. Mixture of methanol and water (56: 44, v/v) was used as a mobile phase at a flowrate of 3 mL/min. After collecting each diasteromer, it was evaporated to dryness below 40^{o}C and re-dissolved in 90% of methanol. The purity of each diasteromer was analyzed by achiral HPLC system with 58% of methanol in water was used as a mobile phase.

Optimum condition for the hydrolysis of labetalol-MBIC diastereomer

Stock solution of labetalol-S-(-)-MBIC diastereomers (1.2 mg/mL) was prepared in 1,4-dioxane, 0.5 mL were placed into distinct 5 mL vials. To this, 0.5 mL of 30 mM H_2SO_4 solution added consider as catalyst and vortexed for 1 min. Then the mixture stirred to decompose diastereomer linkage at 45, 50, 55, 60^{o}C for 48, 56, 60 hours. Proceeding each point of reaction time, the compound was injected to chiral HPLC system for increase peak area of labetalol stereoisomer. And simultaneously decrease of the peak area of labetalol-S-(-)-MBIC diastereomer by achiral analytical HPLC system.

Purification of four stereoisomers After hydrolysis, purification of each labetalol stereoisomer was performed by semi-preparative HPLC system. The mobile phase was a mixture of methanol and water (35 : 65, v/v) with 1 mL/min of flow rate. The labetalol stereoisomer fraction was collected and

evaporated to dryness below 40^{o}C. Chiral purities of them were determined by chiral HPLC using Chirobiotic V column (4.6 mm x 150 mm i.d. 5μm) with methanol: acetic acid: diethylamine (100: 0.3: 0.1, v/v/v) mixture as a mobile phase at a flowrate of 0.5 mL/min monitored at an excitation wavelength of 330 nm and with an emission wavelength of 418 nm [9].

3. Results and discussion

Derivatization of labetalol with a chiral derivatizing reagent (CDR) S-(-)-MBIC reacted with labetalol at hydroxyl groups and produced carbamate derivatives [12]. Optimum reaction condition for derivatization of labetalol and S-(-)-MBIC were achieved at 40^{o}C for 60 min with a good yield (80.9%). Fig. 2 shows the relationship between peak area of diastereomer and reaction time, temperature.

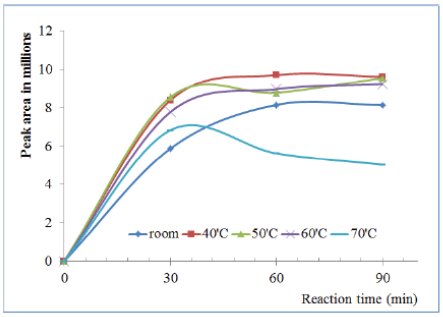

Fig. 2. The effect of temperature and reaction time on derivatization of labetalol and S-(-)-MBIC

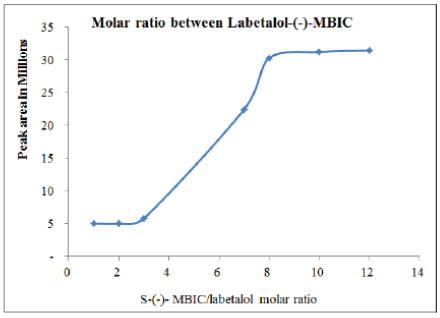

Fig. 3. The effect of S-(-)-MBIC concentration on derivatization reaction

When reaction time increases, peak area also increases. While temperature increases to 700C, peak area of diastereomer decreases induced by S-(-)-MBIC boiling point under 600C. At 400C for 60 min, the value of peak area is the highest. The effect of S-(-)-MBIC concentration added was shown in Fig. 3. Its increase led to a general increase in formation of the diastereomers. Peak area of labetalol-S-(-)-MBIC increase according to the molar ratio and reach a plateu at 1 to 8.

The derivatization of labetalol with S-(-)-MBIC proceeded smoothly to produce four derivatives whose separation could be readily achieved (Rs =1.98) under RP HPLC condition on ODS column (Fig. 4)

Preparation and achiral purity test of labetalol-S-(-)-MBIC diastereomers Each of derivatized stereoisomers was separated and collected by

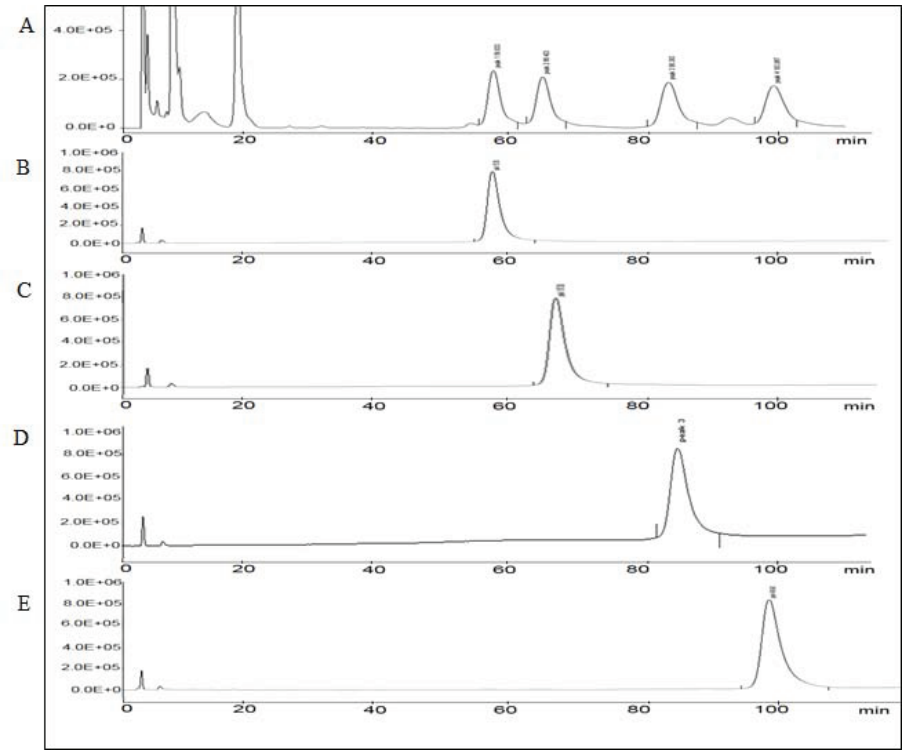

Fig. 4. Analytical chromatograms of labetalol-(-)-MBIC derivatives: (A) reaction of labetalol and S-(-)-MBIC, (B) labetalol-S-(-)-MBIC derivative I, (C) labetalol-S-(-)-MBIC derivative II, (D) labetalol-S-(-)-MBIC derivative III, (E) labetalol-S-(-)-MBIC derivative IV.

semi- preparative reverse phase HPLC, sample size up to 15 mg can be resolved in one injection and 12 mg of samples were separated in a day. The product was light white-colored solid powder. Then, four diastereomers were identified by ^{1}H-NMR, ^{13}C-NMR spectra and checked the purity by achial anaytical HPLC system (see Table 1, and Fig. 4). There is a chiral discrimination between four diastereomers, at the position of carbon number 1, 5, 10.

Table I. Diastereomeric, stereoisomeric purity and overall yields of the method

	Diastereomeric purity (%)[a]	Stereoisomeric purity (%)[b]	Overall Yields (%)[c]
SS-labetalol	99.98	99.95	15.9
RS-labetalol	99.97	99.99	16.3
SR-labetalol	100.00	99.98	16.5
RR-labetalol	100.00	99.97	16.0

[a]Relative purity of diastereomers prepared by semi-preparative HPLC
[b]Relative optical purity of stereoisomer prepared by semi-preparative HPLC
[c]Yields of all processes.

Labetalol-(-)-MBIC derivative I: ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.253 (6H, s, H-9,10), 1.978 (1H, m, H-3), 1.843 (2H, m, H-5), 2.765 (2H, m, H-6), 3.125 (1H, m, H-4), 3.52 (2H, H-2), 4.78 (1H, d, H-8), 4.992 (1H, m, H-1), 7.473 (1H, s, H-7), 6.937-7.33 (12H, m, Ar-H), ^{13}C-NMR (600 MHz, $CDCl_3$) δ: 74.97 (C-1), 50.56 (C-2), 51.32 (C-4), 36.94 (C-5), 22.86 (C-6), 51.22(C-8), 32.6 (C-9), 18.42 (C-10), 118.44 (C-1'), 161.52 (C-2'), 113.08 (C-3'), 124.0 (C-4'), 144.24 (C-5'), 132.44 (C-6'), 133.35 (C-1"), 128.45 (C-2"), 128.79 (C-3"), 127.22 (C-4"), 128.79 (C-5"), 128.45 (C-6"), 140.86 (C-1"'), 125.84 (C-2"'), 128.69 (C-3"'), 126.43 (C-4"'), 128.69 (C-5"'), 125.84 (C-6"').

Labetalol-(-)-MBIC derivative II: ^{1}H-NMR (600 MHz, CDCl3) δ : 1.253 (6H, s, H-9,10), 1.941(1H, m, H-3), 1.810 (2H, m, H-5), 2.709 (2H, m, H-6), 3.131 (1H, m, H-4), 3.535 (2H, H-2), 4.78 (1H, d, H-8), 5.035 (1H, m, H-1), 7.473 (1H, s, H-7), 6.953 - 7.373 (12H, m, Ar-H), ^{13}C-NMR (600 MHz, CDCl3) δ : 74.87 (C-1), 50.39 (C-2), 51.42 (C-4), 37.08 (C-5), 22.87 (C-6), 50.39 (C-8), 32.6 (C-9), 18.3 (C-10), 118.44 (C-1'), 161.55 (C-2'), 113.08 (C-3'), 124.0 (C-4'), 143.98 (C-5'), 132.36 (C-6'), 133.22 (C-1"), 128.34 (C-2"), 128.79 (C-3"), 127.22 (C-4"), 128.79 (C-5"), 128.34 (C-6"), 140.86 (C-1"'), 125.84 (C-2"'), 128.69 (C-3"'), 126.43 (C-4"'), 128.69 (C-5"'), 125.84 (C-6"').

Labetalol-(-)-MBIC derivative III: ^{1}H-NMR (600 MHz, CDCl3) δ : 1.253 (6H, s, H-9,10), 1.8157 (1H, m, H-3), 1.713 (2H, m, H-5), 2.683 (2H, m, H-6), 3.056 (1H, m, H-4), 3.549 (2H, H-2), 4.72 (1H, d, H-8), 4.908 (1H, m, H-1), 7.553 (1H, s, H-7), 6.94 - 7.336 (12H, m, Ar-H), ^{13}C-NMR (600 MHz, CDCl3) δ : 75.57 (C-1), 50.39 (C-2), 51.42 (C-4), 36.0 (C-5), 22.87 (C-6), 50.39 (C-8), 32.6 (C-9), 19.22 (C-10), 118.44 (C-1'), 161.55 (C-2'), 113.08 (C-3'), 124.0 (C-4'), 143.98 (C-5'), 132.36 (C-6'), 133.22 (C-1"), 128.34 (C-2"), 128.79 (C-3"),

127.22 (C-4"), 128.79 (C-5"), 128.34 (C-6"), 140.86 (C-1"'), 125.84 (C-2"'), 128.69 (C-3"'), 126.43 (C-4"'), 128.69 (C-5"'), 125.84 (C-6"').

Labetalol-(-)-MBIC derivative IV: ^{1}H-NMR (600 MHz, CDCl3) δ : 1.253 (6H, s, H-9,10), 1.836 (1H, m, H-3), 1.757 (2H, m, H-5), 2.683 (2H, m, H-6), 3.077 (1H, m, H-4), 3.581 (2H, H-2), 4.728 (1H, d, H-8), 4.995 (1H, m, H-1), 7.581 (1H, s, H-7), 6.94 - 7.373 (12H, m, Ar-H), ^{13}C-NMR (600 MHz, CDCl3) δ : 75.42 (C-1), 50.39 (C-2), 51.42 (C-4), 35.66 (C-5), 22.87 (C-6), 50.39 (C-8), 32.6 (C-9), 19.53 (C-10), 118.44 (C-1'), 161.55 (C-2'), 113.08 (C-3'), 124.0 (C-4'), 143.98 (C-5'), 132.36 (C-6'), 133.22 (C-1"), 128.34 (C-2"), 128.79 (C-3"), 127.22 (C-4"), 128.79 (C-5"), 128.34 (C-6"), 140.86 (C-1"'), 125.84 (C-2"'), 128.69 (C-3"'), 126.43 (C-4"'), 128.69 (C-5"'), 125.84 (C-6"').

Hydrolysis of labetalol-S-(-)-MBIC diastereomer.

The pure labetalol-S-(-)-MBIC diastereomer was hydrolyzed with a simple procedure using 30 mM H_2SO_4 solution as an acidic catalyst. The optimal condition for hydrolysis of four labetalol diastereomers were obtained at 60^{o}C for 56 hours. Peak area of each stereoisomer of labetalol increased according to the time and reached a maximum at 56 hours and no racemization was occurred in this process.

After hydrolysis of the diastereomers, we did not use some complex purification method such as solid phase extraction or liquid-liquid extraction. The hydrolyzed mixture was clean and can be injected to achiral column (Inertsil ODS C18 column (10 mm x 250 mm i.d. 5μm, GL science) to separate and collect pure stereoisomer of labetalol.

Purification and purity test of four stereoisomers

After hydrolysis and purification, chiral purities of four stereoisomers were checked by chiral HPLC system, Carvalho separated the four stereoisomers of labetalol using Chirobiotic V column and reported that elution order of labetalol isomers was (S,R), (S,S), (R,R), and (R,S) labetalol in series [9]. Therefore, hydrolyzed and purified four labetalol stereoisomers were eluted by HPLC system with Chirobiotic V column and could be identified as follow: labetalol-S-(-)-MBIC derivative I, II, III and IV were corresponding with (S,S), (R,S), (S,R), and (R,R) labetalol, respectively. Chiral purities and overall yields of labetalol stereoisomers were at least 99.95% and about 16%, respectively (Table I).

4. Conclusion

For the first time, we reported the indirect method to prepare four stereoisomer of labetalol using a common and inexpensive CDR, S-(-)-MBIC. Stereoisomer of labetalol and S-(-)-MBIC reacted at 40^{o}C for 60 min. Semi-preparative HPLC

was performed to separate and collect each diastereomer, their purities were over 99.97% and yields were about 80.9%. The labetalol-S-(-)-MBIC diastereomer was hydrolyzed with 30 mM H2SO4 solution at 60C for 56 hours. Four labetalol stereoisomers were obtained and purified by achiral semi-preparative HPLC system. The purities and overall yields of them were *leq* 99.95 %, and about 16 %, respectively. This is the simplest and least expensive way of preparative separation by using an ODS column for preparing diastereomer compared to the commercially expensive or time-consuming self-made chiral stationary phase and there is no possibility to occur racemization in hydrolysis reaction.

Acknowledgements The authors thank Pharmaceutical Research Institute and Central Laboratory of Kangwon National University for the use of analytical instrument.

References

[1] C. J. Welch, *Chiral chromatography in support of pharmaceutical process research,* in: G.B. Cox (Ed.), Preparative Enantioselective Chromatography, Blackwell Publishing Ltd., Oxford, U.K., 2005; p. 1-15.

[2] H. Bojarski, H. Y. Aboul-Enein, A. Ghanem, *What's new in chromatographic enantioseparations,* Curr. Anal. Chem., 2005; 1: 59-77.

[3] B. P. Nagori, M. S. Deora, P. Saraswat, *Chiral drug analysis and their application,* Int. J. Pharm. Sci., 2011; 6: 106-113.

[4] W. H. Frishman, *Properties of labetalol, a combined - and - blocking agent, relevant to the treatment of myocardial Ischemia,* Cardiovasc. Drug. Ther., 1988; 2: 343-353.

[5] D. A. Richards, B. N. C. Prichard, *Clinical pharmacology of labetalol,* Br. J. Clin. Pharmac., 1979; 8: 89S-93S.

[6] E. Riva, T. Mennini, R. Latini, *The - and -adrenoceptor blocking activities of labetalol and its RR-SR (50:50) stereoisomers,* Br. J. Pharmacol., 1991; 104: 823-828.

[7] V. A. Maria, R. M. Victorino, *Hypersensitivity immune reaction as a mechanism for dilevalol-associated hepatitis,* Ann. Pharmacother., 1992; 26: 924-926.

[8] J. A. Johnson, W. S. Akers, V. L. Herring, M. S. Wolfe, J. M. Sullivan, *Gender Differences in Labetalol Kinetics: Importance of Determining Stereoisomer Kinetics for Racemic Drugs,* Pharmacother., 2000; 20: 622-628.

[9] T. M. J. P. Carvalho, R. C. Cavalli, M. P. Marques, S. P. Cunha, C. O. Baraldi, V. L. Lanchote, *Stereoselective Analysis of Labetalol in Human Plasma by LC-MS/MS: Application to Pharmacokinetics,* Chirality, 2009; 21: 738-744.

[10] D. M. Desai, J. Gal, *Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents,* J. Chromatogr., 1992; 579, 165-171.

[11] M. Peter, F. Fulop, *Comparison of isothiocyanate chiral derivatizing reagents for high-performance liquid chromatography,* Chromatogr., 2002; 56: 631-636.

[12] K. H. Kim, H. J. Kim, J. H. Kim, J. H. Lee, S. C. Lee, *Determination of the optical purity of (R)-terbutaline by 1H-NMR and RP-LC using chiral derivatizing agent, (S)-(-)-alpha-methylbenzyl isocyanate,* J. Pharm. Biomed. Anal., 2001; 25: 947-956.