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HPLC METHOD FOR ANALYSIS OF CELIPROLOL ENANTIOMERS IN BIOLOGICAL FLUIDS AND PHARMACEUTICAL FORMULATION USING IMMOBILIZED POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASE AND FLUORESCENCE DETECTION

M. HEFNAWY, A. AL-MAJED, A. AL-SUWAILEM , I. AL-SWAIDAN , G.A.E.MOSTAFA*
Pharmaceutical Chemistry Department, College Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

A sensitive and selective high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of celiprolol enantiomers in human plasma, urine, and pharmaceutical formulation. Enantiomeric resolution was achieved on cellulose tris(3,5- dichlorophenyl carbamate) immobilized onto spherical porous silica chiral stationary phase known as Chiralpak IC with a fluorescence detection. The mobile phase consisted of n-hexane:ethanol:triethylamine (70:30:0.4%, v/v/v) has been used with a flow rate of 0.5 mL/min. S-(-)-acebutolol was used as the internal standard. The assay involved the use of acetonitrile for deproteinization of human plasma, and solid phase extraction (SPE) method for human urine samples prior to HPLC analysis. The method was validated in compliance with the international conference on harmonization (ICH) guidelines. The calibration curves were linear over the range of 5-250 ng/mL for each enantiomer. The detection limits of each enantiomer were 1.5 and 2.5 ng/mL in plasma and urine, respectively. The developed method applied for the determination of celiprolol enantiomers in urine, plasma and pharmaceutical formulations. Stress degradation studies as well as assay method indicated that, the method can be used as stability-indicating method and chiral quality control for celiprolol enantiomers by HPLC.

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1. Introduction

Celiprolol, 3-[3-acetyl-4-(3-tert-butyl-amino-2-hydroxy-propoxy) phenyl] -1, 1-diethylurea hydrochloride (Fig .1), is a β_1- adrenergic antagonist with weak vaso - and bronchodilating effects, celiprolol was first introduced in 1985 and has been used as a racemate. Several pharmacokinetic studies[5] have been performed with the racemic drug. In healthy human volunteers, the maximum plasma concentrations ranged from 114- 662 ng/mL after therapeutic oral dose of 200 mg, and the terminal half-lives varied between 3 and 13.6 h, between 3 and 22% of the given racemic celiprolol dose was excreted unchanged in the urine. Metabolic clearance was described as being very low or entirely absent [1-4].

Radioligand binding studies showed that the enantiomers of celiprolol have different pharmacodynamic properties [5] and, therefore, enentiospecific evaluation of the pharmacokinetic is necessarily. Inspite of that except for timolol, all the beta blockers are administered clinically as the racemates. Some of the clinically uses of celiprolol are treatment of hypertension, angina pectoris, supraventricular and ventricular arrhythmias[6].

*Corresponding author: gamal_most@yahoo.com

Most of the celiprolol side effects such as gastrointestinal disturbances, dizziness, depression, asthmatic wheezing are induced by R-(+)- enantiomer and therefore there is a great need to develop quick methods for their enantiomeric resolution [6].

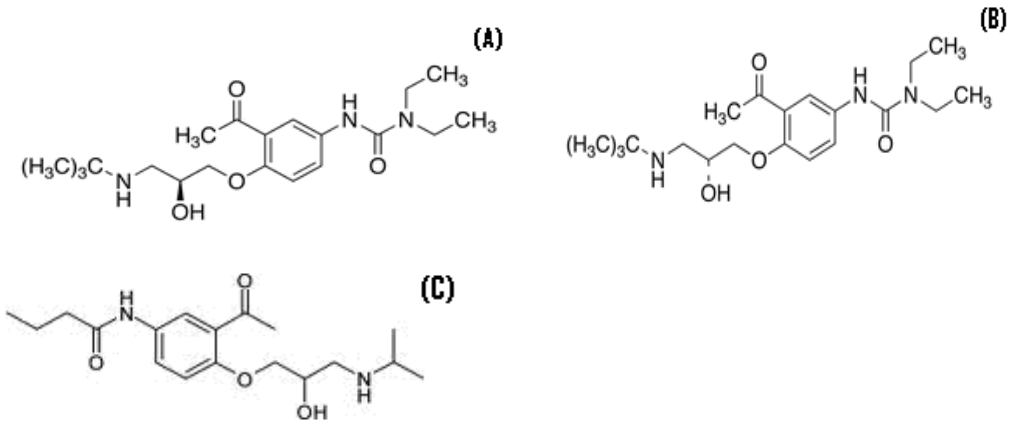

Fig. 1 Chemical structures of S-(-)- (A) and R-(+)- (B)-celiprolol and S- acebutolol(C)

Celiprolol has been analyzed by different analytical techniques including dual-column gas chromatography[7], (nuclear magnetic resonance, spectroscopy, X-ray, differential scanning calorimetry (DSC), and scanning electron microscopy)[8], cyclodextrin capillary zone electrophoresis[9], and non-aqueous chiral cation-exchange capillary electrochromatography[10]. HPLC method of drug analysis has been widely applied for the enantioanalysis of celiprolol using different chiral selectors for examples, β- cyclodextrin CSP[11], Crown tetracarboxylic acid CSP[12], coated type of cellulose tris- 3,5 dimethylphenyl carbamate)[13] both polyacrylamide and S- naphthylethyl carbamate CSPs[14], α- acid glycoprotein CSP[15] and cellulose tris 4-chloro-3-methylphenyl carbamate CSP[16].

Polysaccharide derivatives, being coated or immobilized on silica matrix, have become the first and broadest choice of selectors to be used as chiral stationary phases (CSPs) for both liquid and supercritical liquid chromatography[17, 18].

Three immobilized polysaccharide-derived CSPs have become commercially available namely as, Chiralpak IA, Chiralpak IB, and Chiralpak IC. They are based on tris- (3, 5 dimethylphenyl carbamate) of amylose, tris-(3, 5 dimethylphenyl carbamate) of cellulose, and tris - (3,5 dichlorophenyl carbamate) of cellulose respectively[18-21].

Reviewing the literature revealed that up to present time, nothing has been published concerning analysis of celiprolol enantiomers utilizing Chiralpack IC CSP.

In this work we report the development and validation of celiprolol analysis in human plasma, urine, and tablet using HPLC with fluorescence detection after enantiomeric resolution on Chiralpak IC column.

2. Experimental

2.1. Apparatus and reagents

Chromatography was performed on waters corporation (Milford, MA01757 U.S.A) consisting of 1500 series HPLC pump, and 2475 Fluorescence detector. Data collection and integration was accomplished using DELL computer. The CSP used in this study was the cellulose tris-(3, 5-dichlorophenyl carbamate) which is immobilized on 5μm, 70A silica gel known as Chiralpak IC (250 × 4.6 mm ID) purchased from Chiral Technologies Europe (Cedex, France). The mobile phase was n-hexane: ethanol: triethylamine (70:30:0.4%, v/v/v). The mobile phase was filtered through Millipore membrane filter (0.22 μm) from Nihon, Millipore (Yonezawa, Japan) and degassed before use. The flow rate was 0.5 mL/min with fluorescence detection at 350 and 450 nm for excitation and emission, respectively. (±)- Celiprolol, S-(-) - celiprolol and R-(+) - celiprolol were purchased from Chemie Linz (Linz, Austria). S-(-)- acebutolol was purchased from

Sigma (St- Louis, MO, U.S.A). HPLC grade n-hexane, ethanol and analytical grade triethylamine were purchased from BDH chemicals (Poole, UK). Deionized water purified using a cartridge system (Picotech Water System RTP, NC, USA). Human plasma was obtained from King Khalid University Hospital (Riyadh, KSA) and was kept frozen until use after gentle thawing. Human urine was collected from a healthy male volunteer.

2.2.Preparation of stock and standard solutions

Stock solutions containing 1 mg/mL of individual S-(-)- and R-(+)– celiprolol were prepared in methanol, it purity was found to be 99.8±0.35 by spectrophotometric measurement at 331 nm. Working standard solutions (3μg/mL) were prepared by dilution of individual aliquot of stock solution with the same solvent. The internal standard S-(-) - acebutolol was prepared in methanol to give a concentration of 20 μg mL^{-1} and was further diluted with methanol to give working solution of 2 μg/mL. The solutions were stable for at least seven days if kept in the refrigerator. Appropriate dilutions of the individual working solutions of celiprolol and internal standard were made and used for constructing the calibration curves and spiking both human plasma and urine individually.

2.3.Preparation of quality control samples

The quality control (QC) samples at three concentrations 15, 125 and 200 ng/ mL were prepared by spiking the drug free plasma / urine individually with appropriate volumes of individual S-(-)- and R-(+)- celiprolol and stored frozen until analysis. Before spiking, the drug free (plasma / urine) was tested to make sure that there was no endogenous interferences at the retension time of S-(-)- and R-(+)- celiprolol as well as the retension time of the internal standard S-(-)- acebutolol. The QC samples were extracted with the calibration standards to verify the integrity of the method.

2.4.Assay method for urine and plasma samples

Assay of a human urine sample was performed by placing a 100 μL of urine in to 1.5 mL Eppendrof tube and accurately measured aliquots of 5, 42 and 67 μL of the individual working standard S-(-)- and R-(+)- celiprolol solutions were added. Then 25 μL of the internal working standard solution was added and 200 μL phosphate buffer 50 mM pH 6.8 was added to each tube and diluted to 1mL with acetonitrile and sonicated for 10 minutes to give final concentration of 15, 125 and 200 ng/mL for each enantiomer.

Then each tube was centrifuged at 10000 rpm for 10 minutes, then each sample was applied to C18 (SPE) which previously conditioned by 2 mL methanol and 2 mL phosphate buffer 50 mM pH 6.8, the sample was washed by 250 μl of methanol - Phosphate buffer 50 mM pH 6.8 (20 : 80). Then the C18 SPE was eluted by 250 μL methanol four times, the elute was then sonicated for 10 minutes then evaporated to dryness under gentle air then reconstituted by 1 mL methanol and further sonication was applied then 20 μL of the final solution was injected into HPLC system. Blank human urine samples were processed in the same procedures using acetonitrile instead of celiprolol and acebutolol.

Human plasma sample 300 μL was placed into 1.5 mL Eppendrof tube and accurately measured aliquots of 5, 42 and 67 μL of the individual working standard S-(-)- and R-(+)- celiprolol solutions were added. Then 25 μL of the internal working standard solution was added to each tube and sonicated for 5 minutes then diluted to 1mL with acetonitrile to give final concentration of 15, 125 and 200 ng/mL for each enantiomer then each tube was vortexes for five minutes then centrifuged at 10000 rpm for 10 minutes, the supernatant solution was then evaporated to dryness under gentle air then reconstituted by methanol 1mL, sonicated for 5 minutes then 20 μL of the final solution was injected into HPLC system. Blank human plasma sample was processed in the same procedures using acetonitrile instead of celiprolol enantiomers.

2.5.Preparation of tablet solutions and forced degradation studies

Ten prepared tablets were powdered. An accurately weighed portion equivalent to 3 mg celiprolol was transferred to 100 mL volumetric flask diluted to the mark with methanol. The solution was sonicated for 15 minutes, centrifuged at 3000 rpm for 10 minutes. Accurately

measured aliquots of the supernatant were transferred to three 5 mL volumetric flasks to get the final concentration of celiprolol equivalent to 50, 125, and 225 ng/mL for each enantiomer.

In order to establish whether the analytical method and the assay were stability -indicating, pure active pharmaceutical ingredient (API) of both celiprolol enantiomers was stressed under various conditions to conduct forced degradation studies. The forced degradation was carried out according ICH guidelines[22].

For thermal stress, samples of drug substances were placed in a controlled- temperature oven at 70 ± 2^0C for 7 days, and the resultant solution analyzed every day.

2.6.Validation

The absolute recoveries of each enantiomer from both plasma and urine were calculated by comparing drug peak area ratio of the spiked analyte samples to unextracted analyte of stock solution, which has been injected directly into the HPLC system. Calibration curves were constructed by diluting stock solutions with pooled human plasma or urine to yield six concentrations over the range of 5-250 ng/mL for each celiprolol enantiomer. Linear regression analysis of normalized drug/internal standard peak area ratio versus concentration gave slope and intercept data for each analyte, which were used to calculate the concentration of each analyte in different samples. The intra-day and inter-day (reported as %RSD) and accuracy (reported as % error) of the assay in different samples were determined by assaying three quality control samples in triplicate over a period of 3 days. The concentrations represented the entire range of the calibration curve. The regression equation was used to determine the concentrations in the quality control samples.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

The resolution of celiprolol enantiomers using chiralpack IC is widely affected by several factors. One of the strong factors is the mobile phase used and it's components plus the ratio of that components. The nature of mobile phase can affect enantioselectivity, retension factors, resolution degree and some other system operating parameters, as well as the CSP stability and column life time particularly with that based on Polysaccharide derivatives[23]. Two groups of solvents can be distinguished with regard to the nature of the CSP, are tested.

In principle, the solvents in the first group, denoted as "standard" solvents, can be safely applied on coated (non-immobilized) polysaccharide - based CSPs. These solvents are either non polar solvents, such as hexane and heptane, or polar solvents, such as alcohols and acetonitrile. The coated CSPs, however, are not compatible with the solvents in the "non standard" group [20], solvents of medium polarity, such as tetrahydrofuran (THF), ethyl acetate and chloroform, which may cause a damage of the coated CSP. Once immobilized, the CSP becomes compatible with the whole range of solvents (standard and non - standards) and its enantioselective capacity can be further exploited without comprising the CSP stability[20].

In this study different solvents and different ratio of solvents with or without different ratio of additives (mainly TEA) have been tested, and finally the suitable mobile phase observed is n-hexane: ethanol: TEA (70:30:0.4%, V/V/V) which gave good resolution between the internal standard and both enantiomers (i.e. 4.50 and 2.56).

The importance of ethanol in this mobile phase is to improve peak shape, shortening of retension time and enhancement of selectivity, However, further increase in the ethanol ratio in the mobile phase cause a dramatic increase in the retention time of the drug which may be related to hydrogen bonding between both enantiomers of celiprolol and CSP[20].

On the other hand, TEA (0.4%) used in the mobile phase play an essential role for the separation of celiprolol (basic drug), through suppression of the deleterious effect of residual free silanols on the silica surface which consequently improve the peak symmetry, resolution and selectivity[24].

The mechanisms behind separation of celiprolol using Chiralpak IC column are not fully understood. However, it is may be related to the interactive forces such as π - π interactions, van der Waal forces, hydrogen bonding, and steric effects[23].

The advantage of the proposed method compared with reported method is that the present study is fully validated compared with the published methods (separation)[11, 12-15]. The limit of quantification of the proposed method is almost similar to published methods [13]. Moreover, solid phase extraction was used for extracting the drug from biological sample which is better than those of the reported two-step liquid-liquid extraction method [25]. Another important advantage of the chiral Stationary phase CSP used in this study (Chiralpak IC) is that wide range of polar and non-polar solvent can be used as mobile phase which can give the broad applications scope and the high preparative potential with enhanced robustness and practically unlimited solvent compatibility[24, 26-28].

3.1.1.Linearity

The linear regression analysis of S-(-)- and R-(+)- celiprolol was constructed by plotting the peak area ratio of each enantiomer to the internal standard (Y) versus analyte concentration (ng/mL) in spiked plasma / urine samples (×).

The calibration curve was linear in the range of (5-250) ng/mL for each enantiomer; the lower limit of detections was 1 ng/mL and correlation coefficient (r) of 0.9998 for both enantiomers. A typical calibration curve has the regression of Y= 0.0045× – 0.01 and Y= 0.0056× – 0.03 for S-(-)- and R-(+)- celiprolol enantiomer respectively. The slope (b) were 4.554×10^{-3} and 4.654×10^{-3} while the intercept (a) were -0.01 and -0.03, respectively for S-(-)-and R-(+) celiprolol, respectively**.** The detailed analytical data are shown in Table 1.

Table 1. Validation parameters for the determination of celiprolol enantiomers using the HPLC method.

Parameters	S-(-)- celiprolol	R-(+)- celiprolol
Concentration range ng/ml	5 - 250	5 - 250
Intercept (a)	-0.01	-0.03
Slope (b)	4.55×10^{-3}	5.64×10^{-3}
Correlation Coefficient (r)	0.9998	0.9998
S y/x	3.25×10^{-3}	3.85×10^{-3}
Sa	2.73×10^{-7}	2.87×10^{-7}
Sb	5.28×10^{-6}	6.09×10^{-6}
Limit of quantitation (LOQ) ng/mL	5	5
Limit of detection (LOD)* ng/mL	1	1

* S/N = 6

3.1.2.Precision and accuracy

The accuracy and precision of the results are given with acceptance criteria (inter-day and intra-day % RSD of < 15% and accuracy between 85 and 115%) were met in all cases. The precision and accuracy of the method were determined by using plasma / urine samples spiked at three levels. For plasma samples, the data indicates that intra-day precision and accuracy (N = 6) as expressed by % RSD and % error were 0.83 – 1.94 % and -3.67 - 1.69 % respectively for S-(-)-celiprolol and 0.86 -1.81 % and -4.40 – 1.81 % for R-(+)- celiprolol, respectively. The inter-day precision and accuracy (N = 6) expressed by % RSD and % error 0.85 -1.95 % and -4.27 – 1.62 % respectively for S-(-)- celiprolol and 0.86-1.87 % and -4.00 – 1.96 % for R-(+)- celiprolol, respectively. The detailed analytical data are shown in Table 2.

On the other hand, for urine samples, the data indicates that intra-day precision and accuracy (N = 6) as expressed by % RSD and % error were 0.70 -1.50 % and -1.07 - 1.27 % respectively for S-(-)- celiprolol and 0.99 -1.76 and -1.27 – 1.10 for R-(+)- celiprolol, respectively. The inter-day precision and accuracy (N = 6) expressed by % RSD and % error were 0.68 -1.99 % and -2.87 – 1.67 % respectively for S-(-)- celiprolol and 0.91 -1.92 % and -2.70 – 1.78 % for R-(+)- celiprolol , respectively.

Table 2 Accuracy and precision data for celiprolol enantiomers in spiked human plasma.

Analyte	Actual concen-tration ng /mL	Experimental concentration ng/ mL	Recovery, %	RSD [b],%	% error [C]
a) Intra-day					
S-(-)- celiprolol	15	14.45 + 0.28	96.33	1.94	-3.67
	125	127.12 + 1.55	101.69	1.21	1.69
	200	203.34 + 1.70	101.67	0.83	1.67
R-(+)- celiprolol	15	14.34 + 0.26	95.60	1.81	-4.40
	125	127.16 + 1.50	101.73	1.18	1.73
	200	203.62 + 1.77	101.81	0.86	1.81
b) Inter-day[a]					
S-(-)- celiprolol	15	14.36 + 0.28	95.73	1.95	-4.27
	125	127.03 + 1.53	101.63	1.20	1.62
	200	202.94 + 1.73	101.47	0.85	1.47
R-(+)- celiprolol	15	14.40 + 0.27	96.00	1.87	-4.00
	125	126.89 + 1.49	101.51	1.17	1.51
	200	203.92 + 1.76	101.96	0.86	1.96

[a]Mean + SD n = 6

[b]Expressed as % RSD: (SD/Mean) × 100

[c]Calculated as [(measured concentration – actual concentration)/ actual concentration)] × 100

3.1.3. Selectivity

The selectivity of an analytical method may be defined as the ability to obviously determine the analyte in the presence of additional components such as impurities, degradation products and matrix[24, 28, 29].

The analytical figure of merit for this method is shown in (Table 3). S-(-)- and R-(+)- celiprolol enantiomers were well separated under the HPLC conditions applied**.** Retension times for S-(-)- and R-(+)- celiprolol enantiomers were 20.44 and 23.21 min, respectively.

Table 3. Chromatographic parameter data for celiprolol enantiomers and S-(-)- acebutolol as internal standard.

Analyte	**Rs[a]**	**α[b]**	**k[C]**	**T_R[C]**
S-(-)- acebutolol	4.50	1.50	1.17 $\pm$ 0.02	16.14 $\pm$ 0.05
S-(-)- celiprolol	2.56	1.20	1.76 $\pm$ 0.02	20.44 $\pm$ 0.06
R-(+)- celiprolol	d	d	2.14 $\pm$ 0.03	23.21 $\pm$ 0.08

[a] $R_s = (t_2 - t_1) / 0.5 (W_2 + W_1)$, Where t_2 and t_1 are the retension of the second and the first peaks while Wb_2 and Wb_1 are the half peak width of the second and first peaks.

[b] Separation factor, Calculated as k_2/k_1 where $k = (t_r-t_0)/t_0$, where t_r is the retension of analyte and to is the retension of solvents.

[c] T_R is the retention time, mean $\pm$ SD, n = 10.

[d] Not Calculated.

No interferences were observed in drug free human plasma or urine samples or excipients commonly co-formulated with drug (Figures 2, 3 and 4). Otherwise, there are no peaks detected at the retention time of individual celiprolol enantiomer and internal standard S-(-)- acebutolol.

The selectivity of the method was also evaluated to ensure there were no interference products resulting from forced degradation (Fig.5).

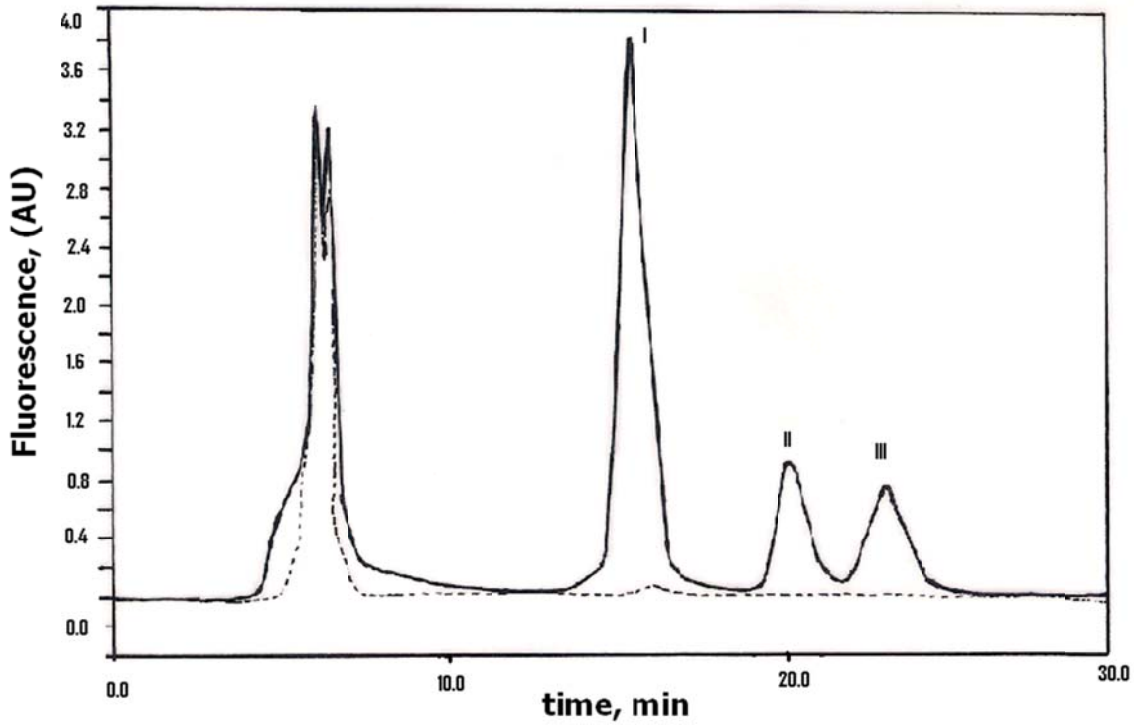

Fig. 2 Chromatograms of (3A.....) blank human plasma and (3B___) spiked with 15ng mL^{-1} of S-(–)- celiprolol (II), R-(+)- celiprolol (III), and 50 ng/mL of S-(–)- acebutolol

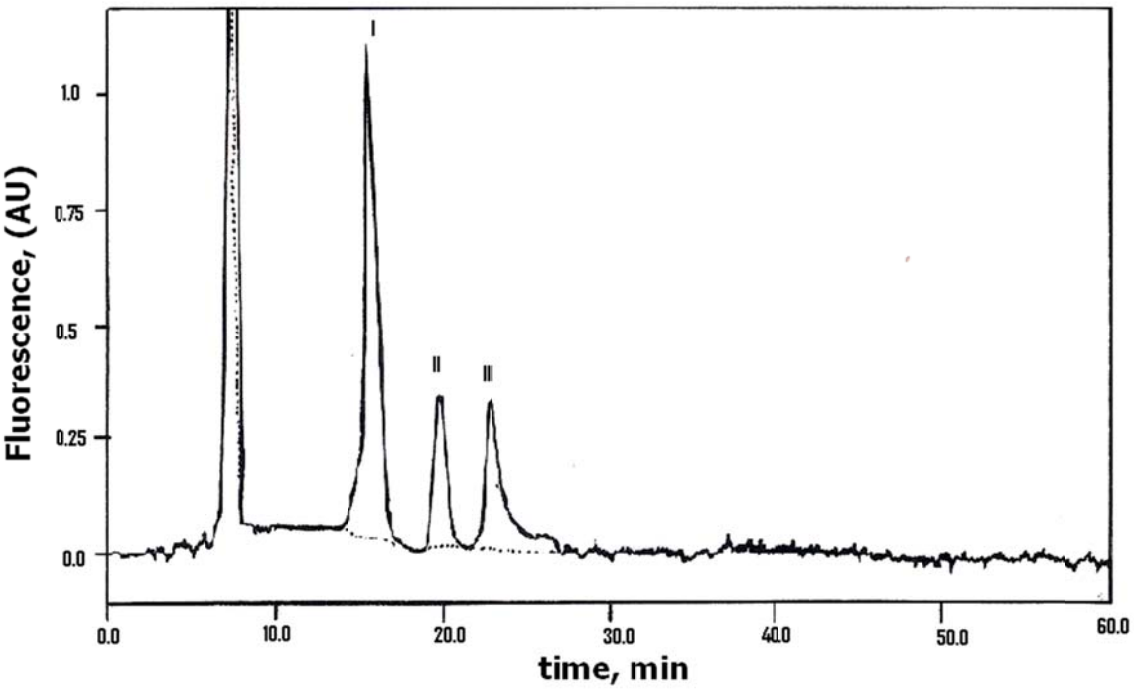

Fig.3 Chromatograms of (4A…..) blank human urine and (4B___) spiked with 15ng/ mL of S-(–) - celiprolol (II), R-(+) - celiprolol (III), and 50 ng/mL of S-(–) -acebutolol.

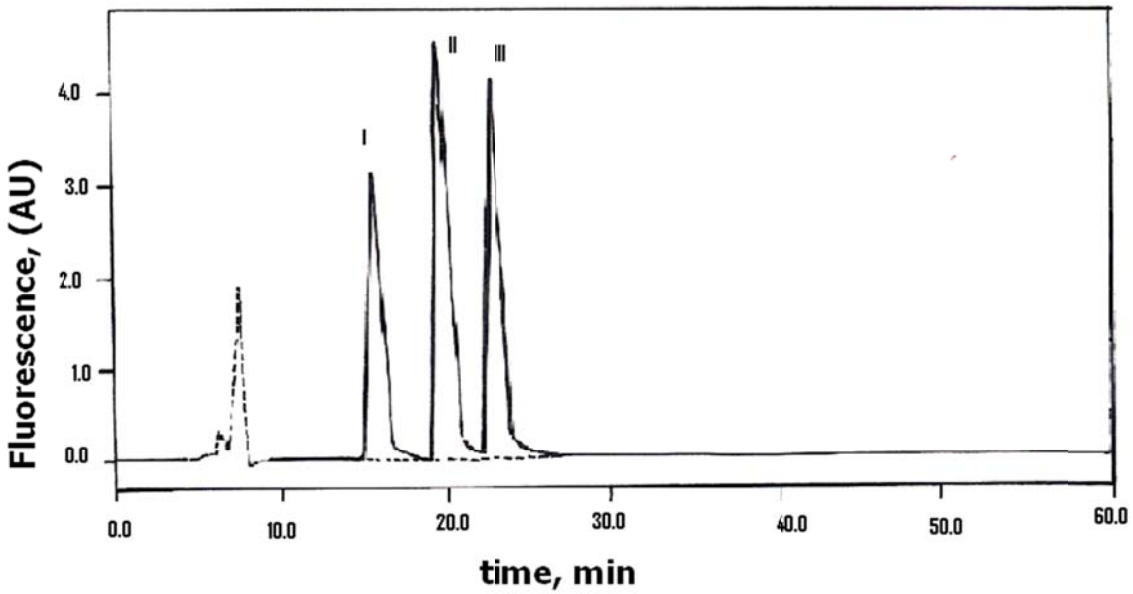

Fig. 4 Chromatograms of (5A……) placebo (excipients), and (5B___) 125 ng/mL of S-(–) - celiprolol II, R-(+)- celiprolol III, and 50 ng/mL of S-(–)- acebutolol (internal standard) recovered from celiprolol tablets.

3.1.4. Limit of detection and limit of quantitation

The limit of detections for each enantiomer of celiprolol in plasma and urine were 1.5 ng/mL and 2.5 ng/mL respectively, while limit of quantitation was 5 ng/mL for both samples.

The good linearity of the calibration graphs and the negligible scatter of experimental points are evident by the values of the correlation coefficient (r) and standard deviation (SD). The correlation coefficient (r) were 0.9998 and 0.9998 and standrda deviations (SD) were 3.25×10^{-3} and 3.85×10^{-3} for respectively for S-(-)-and R-(+) celiprolol, respectively.

3.1.4.Application to pharmaceutical formulations

The validity of the method developed here was applied to various concentrations of celiprolol tablets for determining their contents of celiprolol enantiomers. The values of the overall drug % recoveries and % RSD values of both enantiomers are presented in the (Table 4), indicating that these values are acceptable and the method is precise and accurate.

Table 4. Determination of celiprolol enantiomers tablets prepared in our laboratory.

Enantiomer	Actual concentration ng/mL	Measured concentration ng/mL	Recovery %
S-(-)	50	49.60	99.21
	125	123.58	98.86
	225	225.65	100.29
			99.45+0.75
R-(+)	50	49.65	99.31
	125	123.46	98.77
	225	225.92	100.41
			99.50+0.84

3.1.5. Robustness

The optimum HPLC conditions set for this method have been slightly changed for sample of celiprolol as a mean to evaluate the method robustness. The small changes made in flow rate, and detection wavelengths ensure that the percent recoveries of celiprolol enantiomers were good (it was found the percent recovery 96.15 - 99.45% and 95.42-98.7% for S-and R-celiprolol) under all conditions and remain unaffected by small changes of the experimental parameters. Variations in the experimental parameters provided an indication of its reliability during normal use and concluded that the method conditions were robust.

3.1.6.Forced degradation study

The HPLC studies of 1 µg/mL celiprolol enantiomers on stress testing under different conditions (according ICH guidelines)[25] suggested the following degradation behaviors (Fig. 5). Complete degradation of S-(-)- and R-(+)- celiprolol were found in 1 M, and 0.1 M for HCl, and NaOH respectively (Fig. 5b and 6c). Acid and base treatment with 0.01M concentration for 1h, 24h and 7days was tested against control sample by HPLC technique. The Percent recovery for S- and R-celiprolol were 97.77 % and 97.02 % respectively after 1 h (Fig. 5i), while after 24 h were found to be 93.45 % and 93.48 % respectively (Fig.5j), and after 7 days were 84.13 % and 85.96 % respectively (Fig.6k) at 0.01 M HCl.

On the other hand, the effects of 0.01 M NaOH, percent recovery for S- and R-celiprolol were found to be 97.00 % and 97.42 % respectively at 1 h (Fig.5d), while after 24 h percent recovery remaining were found to be 93.93 % and 94.75 % respectively (Fig.5e), and after 7 days were 87.11 % and 86.87 % respectively (Fig.5f).

S- and R- celiprolol enantiomers were found to be unstable in 3, 5, 10 and 20 % hydrogen peroxide without detection of any degradation products (Fig.5l) which may be related to formation of epoxides group which cannot be detected at the same detection wavelengths or due to cluster formation which mask the degradation products peaks[22, 25, 30-32].

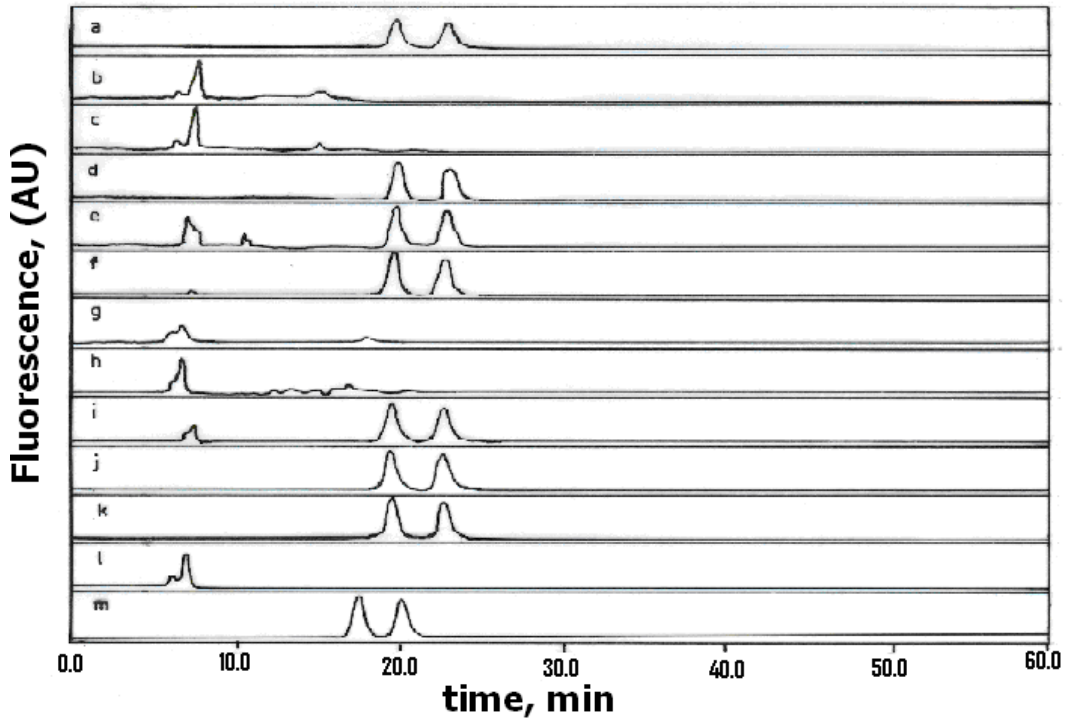

Fig. 5 Overlaid chromatograms of S-(-)- and R-(+)- celiprolol (1µg/mL) at the following conditions:(a) authentic with proposed method, (b) treated with 1M NaOH (1 h reaction time), (c) treated with 0.1M NaOH (1 h reaction time), (d) treated with 0.01M NaOH (1 h reaction time) , (e)treated with 0.01M NaOH (24 h reaction time), (f) treated with 0.01M NaOH (7 days reaction time), (g) treated with 1M HCl (1 h reaction time), (h) treated with 0.1 M HCl (1 h reaction time), (i) treated with 0.01 M HCl (1 h reaction time), (j) treated with 0.01 M HCl (24 h reaction time), (k) treated with 0.01 M HCl (7 days reaction time), (l) treated with 3% H_2O_2 (1 h reaction time), and (m)thermal stability at 70^0C for 7 days

Day stability of both celiprolol enantiomers also has been tested. It was found that both enantiomers of celiprolol are stable with percent remaining ranged from 98.45 to 99.73 %.

The thermal stability of celiprolol enantiomers also has been evaluated. The freshly prepared solution at room temperature and the 7 days stored sample at 70 ± 2^0C, were analyzed by the proposed HPLC method. The concentrations of celiprolol enantiomers in stored sample were calculated and compared to that present in the freshly prepared sample. From these results, we can conclude that there were no degradation products at elevated temperature and the drug is stable at 70 ± 2^0C for 7 days (Fig.5m) indicating the possibility of using celiprolol samples over a period of 7 days at 70°C without degradation. However, the retension times of S-(-)- celiprolol and R-(+)-celiprolol have been slightly changed from 20.44 and 23.21 minutes to 18.83 and 21.66 minutes respectively which considered as the usual temperature effect (where some samples elute more rapidly at higher temperatures)[33].

4.Conclusions

An enantioselective HPLC method that enables sensitive determination of both celiprolol enantiomers in all human plasma, urine, and tablet was developed. The current study indicated that, this method can be used as stability indicating assay as well as for potency assay of both celiprolol enantiomers from drug products. The total run time for this method is 25 min, which allows processing of over 55 samples per day. This method has good sensitivity, precision, selectivity, robustness, and reproducibility.

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