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Original Article

DEVELOPMENT AND VALIDATION OF BIOANALYTICAL HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND NEBIVOLOL IN HUMAN PLASMA

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ABSTRACT

Objective: To develop and validate a modified isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method for determination of cilnidipine and nebivolol in human plasma to be used for pharmacokinetic studies.

Methods: The drug was extracted from plasma samples by direct protein precipitation technique using acetonitrile. Amlodipine was used as internal standard (IS). Samples were analyzed on BDS C_{18} column (250 x 4.6 mm, 5 µm), applying ortho phosphoric acid (0.1%): Acetonitrile, at a ratio of 45:55 v/v in isocratic mode as a mobile phase at a flow rate of 1 ml/min to attain adequate resolution. Separations were performed at room temperature and monitored at a wavelength of 260 nm after injection of 50µl samples into the HPLC system. The analytical method was validated according to FDA bioanalytical method validation guidance. The method was applied for pharmacokinetic study of cilnidipine and nebivolol tablets-10 mg and 5 mg were administered as a single dose to 6 healthy male rabbits under fasting condition. Twelve blood samples were withdrawn from each rabbit over 24 h periods. From the plasma concentration-time data of each individual, the pharmacokinetic parameters; Cmax, Tmax, AUC0-t and AUC0-∞ were calculated.

Results: A peak area was obtained for cilnidipine and nebivolol at 3.943 and 4.719 min retention time respectively. Linearity was established at a concentration range of 0.20-20 µg/ml (r^2=0.999, n=8) for cilnidipine and 0.02-2 µg/ml (r^2=0.999, n=8) for nebivolol. The lower limit of quantitation (LLOQ) was identifiable and reproducible at 0.2µg/ml for cilnidipine and 0.02 µg/ml for nebivolol. The coefficients of variation (%cv) of the intra-day and inter-day precision of cilnidipine at 600, 1000 and 1600ng/ml levels were found to be 6.90%, 6.19%, 5.22%; and 7.74%, 6.54%, 5.77%, respectively, which are lower than the accepted criteria limits (15-20 %). The mean recovery (%) cilnidipine at 600, 1000, and 1600ng/ml was found to be 101.03%, 99.27% and 104.87%, and for nebivolol 60, 100, and 160 ng/ml was found to be 106.13%, 107.03% and 98.06% respectively. Stability at different conditions and in autosampler was also established. The mean pharmacokinetic parameters; Cmax, Tmax, AUC0-t and AUC0-∞ were 6 ng/ml, 2 hr, 96.76 mg. hr/ml, 63.45 mg. hr/ml for cilnidipine and 5.8ng/ml, 2hr, 74.78 mg. hr/ml, 100.25 mg. hr/ml for nebivolol respectively.

Conclusion: The present analytical method was found to be specific, sensitive, accurate and precise for quantification of cilnidipine and nebivolol in human plasma. It can be successively applied for pharmacokinetics, bioavailability and bioequivalence studies.

Keywords: Cilnidipine, Nebivolol, HPLC, Human plasma, Pharmacokinetics

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INTRODUCTION

Cilnidipine 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-carboxylic acid 2-methoxyethyl(2e)-3-phenyl-propenyl ester (fig. 1)is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long-lasting vasodilating effect. Cilnidipine is used in the treatment of hypertension. Cilnidipine shows first pass mechanism. Cilnidipine is used in combination with others drugs like telmisartan, olmesartan. Cilnidipine and its formulations are not official in any pharmacopoeias [2]. Nebivolol hydrochloride is a β1 receptor blocker. It is chemically (1rs, 1'rs)-1,1'-[(2rs, 2'sr)–bis (6–flurochroman-2-yl)]-2, 2'-iminodiethanol hydrochlo-ride (fig. 2). Nebivolol and cilnidipine are used alone in treatment of hypertension but when they are given in combination synergistic action obtained and hence dose is reduced.

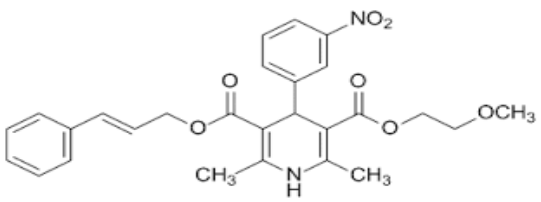

Fig. 1: It shows chemical structure of cilnidipine

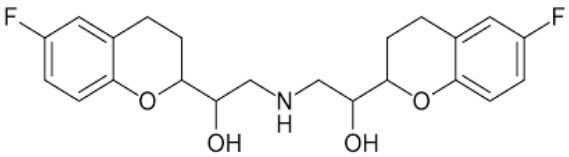

Fig. 2: It shows chemical structure of nebivolol

To date several methods have been developed for the quantification of cilnidipine and nebivolol either alone or in combination with other drugs in different matrices [1-3]. HPTLC method reported for determination of nebivolol hydrochloride and cilnidipine in combined tablet dosage form [4]. The simultaneous quantification of cilnidipine combinations with olmesartan medoxomil, chlorthalidone, and valsartan in oral formulations were also reported [5-8]. Nevertheless, there is still an increasing interest for development of more specific, accurate, precise and rapid method for determination of cilnidipine and nebivolol especially in human plasma. The aim of this study was to develop and validate HPLC analytical method for determination of cilnidipine [9] and nebivolol in human plasma in order to be utilized for studying the pharmacokinetics of cilnidipine and nebivolol after a single oral dose of LNBETA tablets 10 mg and 5 mg.

MATERIALS AND METHODS

Materials and reagents

Waters HPLC equipped with Inertsil ODS C_{18} (250×4.6×5µ), column, solvents and buffers of HPLC grade purchased from Merck Ltd. API were procured as gift samples from Spectrum labs, Hyderabad and formulation LNBETA was purchased from local market, Hyderabad.

Instruments

Waters 2695 alliance HPLC system, Waters 2996 PDA detector. Empower-2 software (USA). Stuart scientific vortex shaker (England).

Preparation of standard solutions

Stock solution of cilnidipine (10 mg/ml) and nebivolol (1 mg/ml) was prepared in diluent (water: acetonitrile 50:50) using cilnidipine and nebivolol standard powders. The internal standard stock solution of amlodipine (46µg/ml) was prepared in diluent using amlodipine standard powder. Working standard solutions of cilnidipine, nebivolol and amlodipine were prepared by dilution of their respective stock solution with a mixture of acetonitrile: water (50:50) to produce a final concentration of 10000 µg/ml and 1000 µg/ml respectively.

Standard calibration curve preparation

Standard solutions of cilnidipine and nebivolol were prepared by serial dilution of working solution (100µg/ml) with a mixture of acetonitrile: water (50:50) to attain a concentration of 46, 92, 138, 1150, 1725, 2300, 3680 and 4600 µg/ml for cilnidipine and 4.2**,** 10.08**,** 13**,** 115**,** 170**,** 230**,** 360 and 450µg/ml for nebivolol Amlodipine was used as internal standard which has been taken at a concentration of 46µg/ml in each level. All solutions were prepared daily.

Plasma sample preparation for calibration curve

Human blood samples were transferred to heparinized tubes and then centrifuged at 4000rpm for 10 min. The Plasma was separated by polypropylene disposable tips and stored at-20 °C±2 in the deep freezer until analysis.

The calibration curve of cilnidipine and nebivolol in plasma was constructed by spiking 900 µl of plasma samples (which was first thawed at room temperature) with 100 µl of the previously prepared standard solutions (0.5, 1.0, 2.0, 5.0, 10.0 and 15.0µg/ml, keeping internal standard amlodipine at a concentration of 10µg/ml in each one). Accordingly, the plasma samples contain a final concentration of cilnidipine and nebivolol equivalent to 46, 92, 138, 1150, 1725, 2300, 3680 and 4600 µg/ml for cilnidipine and 4.2, 10.08, 13, 115, 170, 230, 360, and 450 µg/ml, for nebivolol respectively. Amlodipine was used as internal standard which has been taken at a concentration of 460ng/ml in each. While blank plasma samples were spiked with 100µl of a mixture of acetonitrile: water (1:1). The samples were vortex to mix for 30 seconds to be applied to the extraction and analytical procedure.

Quality control (QC) samples preparation

Standard solutions of cilnidipine and nebivolol were prepared by serial dilution of working solution (100µg/ml) with mixture of acetonitrile: water (50:50) to attain a concentration of 0.600, 10.00 and 16.00µg/ml for cilnidipine and 0.060, 1.000 and 1.600µg/ml for nebivolol QC samples preparation, keeping amlodipine internal standard at a concentration of 4.6µg/ml in each. All solutions were prepared daily. Quality Control plasma samples were prepared by spiking 900 µl of plasma samples (which was thawed at room temperature) with 100 µl of the freshly prepared standard solutions (0.60, 10.0 and 16.00 µg/ml of cilnidipine and 0.060, 1.000 and 1.600 µg/ml of nebivolol; with 4.6µg/ml of amlodipine). Accordingly plasma samples contain a final concentration of cilnidipine and nebivolol equivalent to 600, 1000, 1600ng/ml and 60,100,160ng/ml respectively and 460ng/ml of amlodipine as internal standard.

Sample preparation for HPLC injection

Drug was extracted from plasma samples using direct protein precipitation technique. 2 ml of acetonitrile: water (50:50) was added to 250 µl of plasma, 50 µl of internal standard, 10 µl of cilnidipine and 10 µl of nebivolol spiked plasma samples. The samples were shaken and then centrifuged at 3200rpm for 20 min. Finally 50 µl of the clear supernatant were injected into the HPLC column.

Chromatographic conditions

The different HPLC experimental parameters were optimized. The optimized chromatographic conditions were column: BDS C18 column (250 x 4.6 mm, 5 µm), mobile phase: ortho phosphoric acid (0.1%) buffer: acetonitrile (45:55, v/v), detection: UV detector set at a wavelength λ_{max} of 260 nm, flow rate: 1.0 ml/min, injection volume: 50 µl, auto sampler temperature: ambient.

The mobile phase was always degassed and clarified by filtration through porous membranes with 0.45 µm pore size. A mobile phase degasser was connected on line during the analysis runtime, and then pumped at a flow rate of 1 ml/min, in isocratic mode on the column. The sample (50µl) was injected into HPLC system and the data was acquired employing Empower-2 software.

Method validation

The analytical method was validated according to ICH guidelines [15-17] with respect to the following parameters:

Calibration and linearity

The linearity of the method was established from the standard calibration curve constructed at several concentration levels of 0.2-20 µg/ml and 0.02 to 2 µg/ml for cilnidipine and nebivolol respectively. Triplicate 50 µl injections were made for each working standard solution. The peak area for each concentration was recorded and then plotted against the corresponding concentration to obtain the calibration graph. In addition, a blank and a zero sample were prepared to confirm the absence of interferences.

Selectivity/specificity

The Selectivity/specificity was evaluated by extracting different blank plasma samples. The absence of interfering peaks at the same retention time of analytes or internal standard (Amlodipine) was considered as evidence for selectivity/specificity

Accuracy and precision

Intra-day accuracy and precision

The intra-day precision and accuracy of the assay were measured by analyzing five spiked samples of cilnidipine and nebivolol at three different concentrations (600, 1000 and 1600ng/ml and 60,100 and 160ng/ml); the concentrations were calculated by using the regression equation of the calibration curve. The deviation of the mean from the true value serves as the measure of accuracy. The precision and accuracy deviation values should be less than 15% of the actual values except at lower limit of quantitation (LLOQ) where it shouldn't deviate>20%. The statistical evaluation includes mean, standard deviation (SD), coefficient of variation (%CV) and accuracy.

Inter-day accuracy and precision

The inter-day precision were done at three different concentrations (600, 1000 and 1600ng/ml and 60,100,160ng/ml) over three days, the concentrations were measured by analyzing samples of five determinations from each concentration per day and were calculated applying the regression equation of the calibration curve. The statistical evaluation includes mean, SD, % CV and accuracy.

Accuracy and precision for quality control (QC) samples

The accuracy and precision for QC samples were demonstrated by analyzing over two days duplicates of QC sample at three concentration levels representing the entire range of the standard calibration curve. The low QC samples (600ng/ml,60ng/ml) were designed to be three times the LLOQ (200ng/ml, 20ng/ml), while the mid QC samples were taken at the center (1000ng/ml,100ng/ml) and the high QC samples were taken near the upper limit of quantitation (ULOQ) which is (1600ng/ml,160ng/ml).

Recovery

It can be calculated by comparison of the analyte response after sample workup with the response of a solution containing the analyte at the theoretical maximum concentration.

The absolute recovery was calculated for cilnidipine, nebivolol and internal standard by comparing peak areas of the extracted samples with the un-extracted pure authentic standard solutions peak areas The relative recovery was determined for cilnidipine and nebivolol by comparing the calculated concentrations of extracted samples to their respective nominal values. Both absolute and relative recoveries of cilnidipine and nebivolol were measured at three concentration levels (600, 1000, 1600ng/ml and 60,100,160ng/ml).

Sensitivity

The lowest concentration in the calibration curve was considered as the LLOQ and should meet the following criteria; LLOQ response is five times the response of the blank, LLOQ response is identifiable, discrete and reproducible with precision of 20% and accuracy of 80-120%.

The peak was identifiable, precise and accurate at this concentration. The LLOQ of cilnidipine and nebivolol in plasma was considered to be 200ng/ml and 20ng/ml.

Stability studies

The stability of the cilnidipine and nebivolol in solutions and plasma samples was also evaluated during method validation. Cilnidipine and nebivolol stability was evaluated using two concentration levels (low and high quality control, corresponding to 600,1600ng/ml and 60,160ng/ml respectively). The stability of cilnidipine and nebivolol was also evaluated in post extracted samples kept in the auto sampler at 10 °C for 60 h, as well as in plasma samples kept at freezer and after being stressed to 3 freeze-thawing cycles (24 h each cycle). All samples described above were compared to freshly prepared cilnidipine and nebivolol samples at the same concentration level.

Pharmacokinetic study in rabbits

The method described above was applied to quantify the plasma concentration of cilnidipine and nebivolol in a single-dose pharma-cokinetic study [11-14] conducted on six white male albino rabbits. The protocol was approved by the institutional ethical committee at the Krishna teja pharmacy college, Tirupati, India and approval number was KTPC/IAEC/Ph. D/2015/I. The experiments were conducted as per CPCSEA guidelines. The rabbits weighing 2.5±0.3 kg were housed with free access to food and water, except for the final 12 h before experimentation. The rabbits were divided into 2 groups of 6 rabbits each with cross over technique (n=6). All rabbits were fasted overnight, access to water during the experiment and the animals were fed 24 h after the oral dose. One group of animals received a single dose of pure drugs, formulated as a suspension containing sodium carboxy methyl cellulose. The second group was administered a solution containing marketed formulation at the same dose. 2.5 ml of blood samples were collected from the marginal ear vein at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12 and 24 hr time points into heparinized collection tubes. The blood was immediately centrifuged for 10 min at cooling temperature. The supernatant plasma layer was separated and stored at-20 °C until analyzed. The plasma samples were analyzed for cilnidipine and nebivolol concentrations as described above. The total area under the observed plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule. The first order elimination rate constant (K_e) was estimated by the least square regression of the points describing the terminal log-linear decaying phase. $t_{1/2}$ was derived from K_e ($t_{1/2}$ ¼ln 2/K_e). The absorption rate constant (K_a) was determined by residual method. The maximum observed cilnidipine and nebivolol concentration (c_{max}) and the time at which c_{max} was observed (t_{max}) were reported directly from the profile.

RESULTS AND DISCUSSION

Linearity

The linearity of the method was evaluated from the calibration curve of spiked plasma samples at several concentration levels of cilnidipine and nebivolol (constructed for six consecutive days). The mean area (ratio of peak area of the drug to the peak area of the internal standard) yielded a linear correlation over a concentration range of 0.2µg/ml to 20µg/ml and 0.02µg/ml to 2µg/ml respectively. The method exhibited excellent linearity for this range. A typical calibration curve of spiked plasma samples with the regression equation and their respective correlation coefficient (r^2) of cilnidipine and nebivolol were shown in (fig. 3 and fig. 4). The average correlation coeffient was found to be 0.999 for both drugs with goodness of fit.

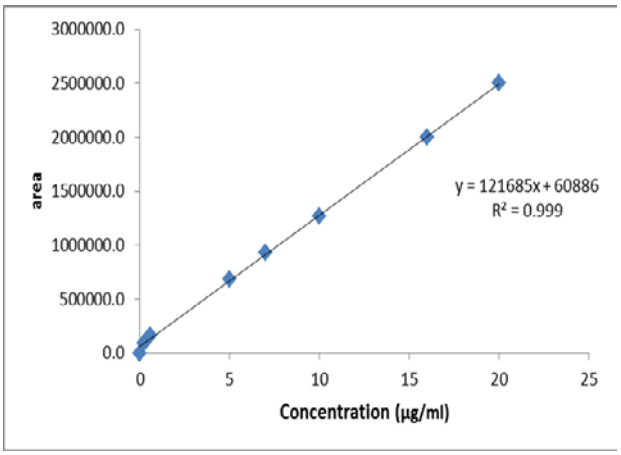

Fig. 3: It shows calibration curve of cilnidipine with amlodipine as internal standard in human plasma

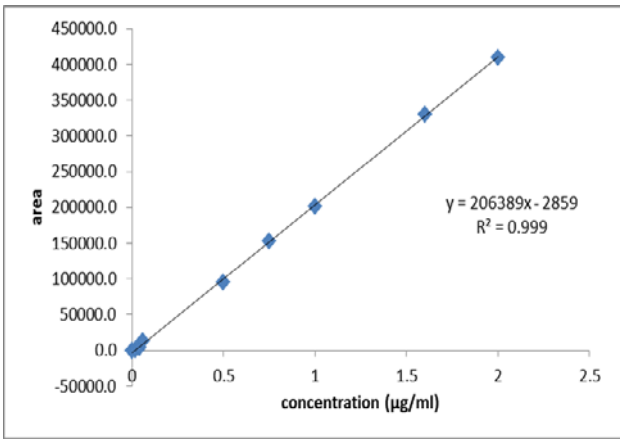

Fig. 4: It shows calibration curve of nebivolol with amlodipine as internal standard in human plasma

Specificity/selectivity

Representative chromatogram of blank plasma confirmed there is no significant interference from the endogenous component as shown in (fig. 5). Chromatograms of spiked plasma samples of cilnidipine and nebivolol at concentration of 200ng/ml and 20ng/ml respectively with the internal standard amlodipine at a constant concentration (460ng/ml) confirming that cilnidipine, nebivolol and amlodipine were well resolved and completely separated at retention times of 3.976,4.835 and 5.794 min, respectively as shown in (fig. 6, 7).

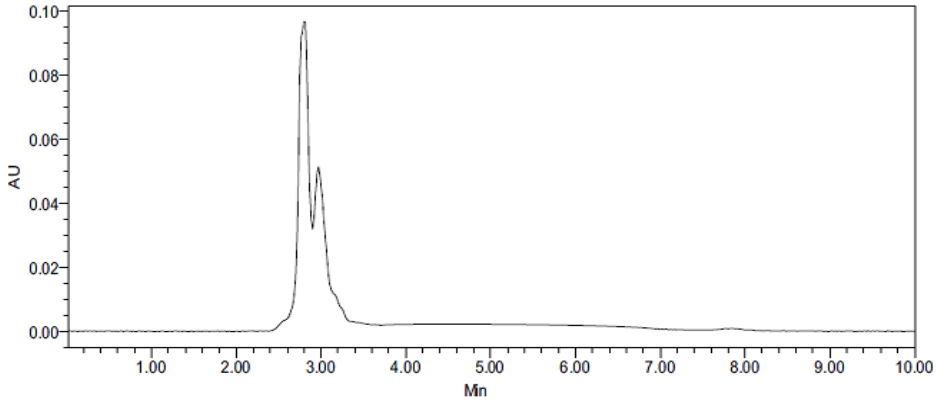

Fig. 5: It shows HPLC chromatogram of a blank human plasma sample

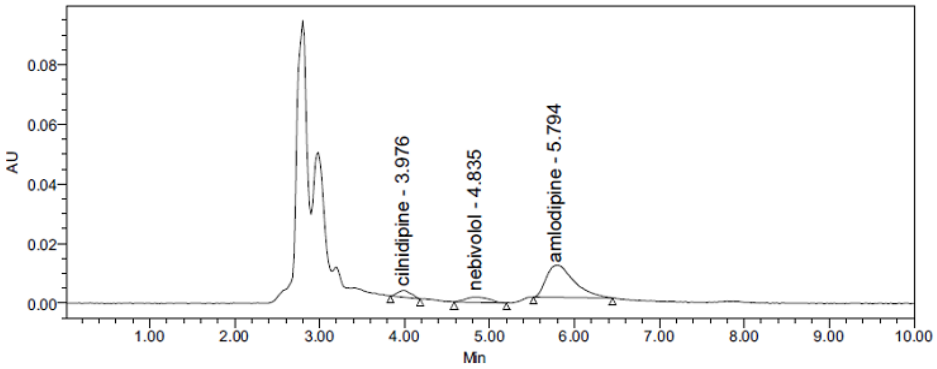

Fig. 6: It shows HPLC chromatogram representing complete resolution of the internal standard (IS) amlodipine peak from cilnidipine (200ng/ml) and nebivolol (20ng/ml) peak at a retention time 5.794, 3.976 and 4.835 min, respectively

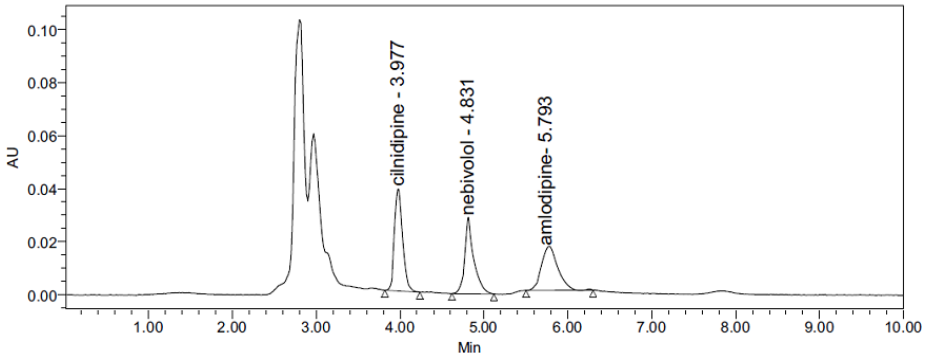

Fig. 7: It shows HPLC chromatogram representing complete resolution of the internal standard (IS) amlodipine peak (460ng/ml) from cilnidipine peak (1600ng/ml) and Nebivolol (160ng/ml) at a retention time 3.9 and 4.8 min, respectively

Accuracy and precision

Intra-day accuracy and precision

Intra-day accuracy of the method for cilnidipine and nebivolol were ranged from 99.27% to 104.87% and 98.06% to 107.03% respectively. While the intra-day precision ranged from 5.22% to 6.90% at the concentrations of 600, 1000 and 1600ng/ml for cilinidipine and 7.24% to 11.49 % at the concentration of 60,100,160ng/ml for nebivolol.

The results were presented in (table 1).

Table 1: It shows intra-day precision and accuracy for cilnidipine and nebivolol in spiked human plasma samples

	Cilnidipine concentration in human plasma			Nebivolol concentration in human plasma		
	1600ng/ml	**1000ng/ml**	**600ng/ml**	**160ng/ml**	**100ng/ml**	**60ng/ml**
Mean*	16.1653	9.9265	0.6292	1.6980	1.0703	0.0588
±SD	1.11470	0.61472	0.03286	0.13672	0.09527	0.00426
Precisions as CV%	6.90	6.19	5.22	8.05	8.90	7.24
Accuracy%	101.03	99.27	104.87	106.13	107.03	98.06

*3 determinations

Inter-day accuracy and precision

Inter-day accuracy of the method for cilnidipine and nebivolol were ranged from 97.72% to 102.93% and 97.87% to 105.41 % respectively. While the inter-day precision ranged from 5.77% to 7.74% at the concentrations of 600, 1000 and 1600ng/ml for cilinidipine and 7.41 % to 7.88% at the concentration of 60,100,160ng/ml for nebivolol. The results were presented in (table 2).

Table 2: It shows inter-day precision and accuracy for cilnidipine and nebivolol in spiked human plasma samples

	Cilnidipine concentration in human plasma			Nebivolol concentration in human plasma		
	1600ng/ml	1000ng/ml	600ng/ml	160ng/ml	100ng/ml	60ng/ml
Mean*	15.6711	9.7723	0.6176	1.6736	1.0541	0.0587
±SD	1.21247	0.63934	0.03561	0.13195	0.08591	0.00435
Precisions as CV%	7.74	6.54	5.77	7.88	8.15	7.41
Accuracy%	97.94	97.72	102.93	104.60	105.41	97.87

*3 determinations

Accuracy and precision for quality control (QC) samples

Quality control samples were analyzed for cilnidipine and nebivolol at the three levels 600, 1000 and 1600ng/ml and 60, 100, and 160ng/ml. The results were shown in (table 3). The accuracy and precision around the mean value did not exceed 15% of CV. Hence the developed method met the limits of accuracy and precision.

Table 3: It shows accuracy and precision for cilnidipine and nebivolol quality control samples

	Cilnidipine concentration in human plasma			Nebivolol concentration in human plasma		
	QC Low (600ng/ml)	QC Mid (1000ng/m)	QC High (1600ng/m)	QC Low (60ng/ml)	QC Mid (100ng/m)	QC High (160ng/ml)
Mean*	164552	1252583	1958595	12513	202344	327466
±SD	1061.40	16570.99	33214.31	70.65	1284.56	2232.86
Precisions as CV%	0.65	1.32	1.70	0.56	0.63	0.68
Accuracy%	73.22	67.57	76.65	61.31	76.20	77.00

*3determination

Recovery

The absolute and relative recovery determined for cilnidipine and nebivolol shown to be consistent, precise and reproducible at the three levels 600, 1000 and 1600ng/ml and 60, 100, 160ng/ml.

The data was depicted in (table 4). While, the absolute recovery of amlodipine (IS) was found to be 69.572%.

Table 4: It shows data for sensitivity of lower limit of quantitation (LLOQ)

Concentration	Actual concentration (ng/ml)	Accuracy %	Mean (ng/ml)*	CV%
Cilndipine 200ng/ml	0.185	100.00	0.2000	8.79
	0.210			
	0.196			
	0.231			
	0.188			
	0.190			
Nebivolol 20ng/ml	0.019	100.00	0.0200	7.07
	0.020			
	0.021			
	0.022			
	0.018			
	0.020			

*6 determinations

Sensitivity

The sensitivity of the method was established at 200ng/ml (LLOQ) of cilnidipinde with % CV of 8.79 and 2ng/ml (LLOQ) of nebivolol with % CV of 7.07. The data for LLOQ was presented in table 4. The chromatogram of an extracted plasma sample spiked with 200ng/ml and 20ng/ml of cilnidipine and nebivolol were shown in (fig. 6).

Matrix effect

The assessment of matrix effect constitutes an important and integral part of validation for quantitative methods for supporting pharmacokinetics studies. It was performed by processing plasma samples in triplet (n = 3). LQC and HQC working solutions were spiked post extraction in duplicate. The results obtained were well within the acceptable limits, as the %RSD of the area ratios of post spiked recovery samples at LQC were 7.31 and at HQC were 7.50 for cilnidipine and at LQC were 7.78 and at HQC were 11.2 for nebivolol which were within 10%. Hence minor suppression or enhancement of analyte signal due to endogenous matrix interferences did not affect the quantification of analytes and IS peak.

Stability studies

Stability studies were performed to evaluate the stability of cilnidipine and nebivolol both in aqueous solution and in plasma after exposing to various stress conditions. The stability studies performed include stock solution stability of cilnidipine, nebivolol and amlodipine in

stock solution, stock dilution stability of cilnidipine and nebivolol in dilutions, bench top stability in plasma, free thaw stability in plasma, long term storage stability in plasma and auto sampler stability of processed samples. All stability evaluations were performed as per international regulatory guidelines.

Cilnidipine, nebivolol and amlodipine stock solutions (1 mg/ml) remained stable when stored at refrigerator conditions for 7 d including the storage at room temperature for 8 h. Cilnidipine and nebivolol were stable in plasma samples when stored at room temperature for 18 h. Cilnidipine and nebivolol were found to be stable for 3 freeze and thaw cycles. Cilnidipine and nebivolol were stable and did not show any degradation when stored in the freezer for 85 d. Cilnidipine and nebivolol in the processed samples were stable for 60 h when stored in the auto sampler at 10 °C.

Application of the present validated method of analysis for pharmacokinetic study of cilnidipine and nebivolol in human plasma

Application

The validated method has been successfully used to quantitate cilnidipine and nebivolol concentrations in rabbits, after administration of a single combination dosage form (Tablet containing 10 mg of cilnidipine and 5 mg of nebivolol as an oral dose). The statistical data evaluated were Cmax (maximum observed drug concentration during the study), AUC0-t (area under the plasma concentration–time curve measured to the last quantifiable concentration, using the trapezoidal rule), AUC0∞ (AUC0-t plus additional area extrapolated to infinity, calculated using the formula AUC0-t+Ct/Kel, where Ct is the last measurable drug con-centration), Tmax (time to observe maximum drug concentration). The mean Cmax data obtained, justified the LOQ levels selected, as they are at least less than five half-lives of the obtained Cmax values. The mean Cmax observed for test and reference of cilnidipine 6.2 and 6.0ng/ml and 5.6 and 5.8ng/ml for nebivolol (fig. 8 and 9). The corresponding mean Tmax for cilnidipine and nebivolol, for test and reference formulations were 2.0 h. The mean AUC0-t test and reference formulations were for cilnidipine 65.57 and 63.45 ng x h/ml and for nebivolol, 74.59 and 74.78 ng x hr/ml, respectively while AUC0–inf were 1740 and 1844 ng x hr/ml, respectively. The 90% confidence intervals of the ratios of means Cmax, AUC0-t and AUC0 ∞ all fell within the acceptance range of 0.8–1.25, demonstrating the bioequivalence of the two formulations of cilnidipine and nebivolol.

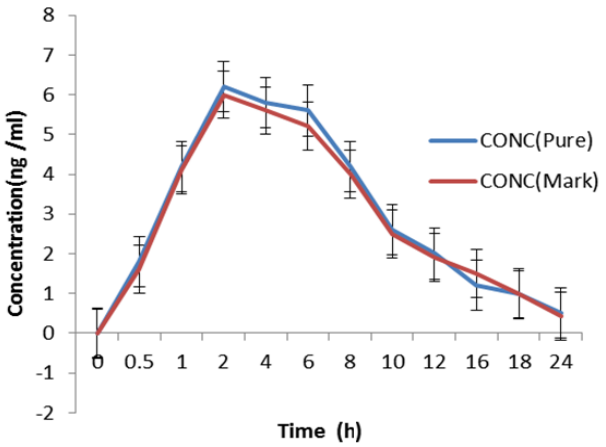

Fig. 8: It shows mean plasma concentrations (mean±SD) of cilnidipine versus time profile obtained after a single dose of LN BETA 10 mg+5 mg tablet administered to 06 healthy male rabbits

CONCLUSION

Although, many other researchers have provided breakthrough methods for quantification of cilnidipine and nebivolol individually, as can be seen from the references listed. We provide simple, specific, sensitive and rapid method for the simultaneous estimation of cilnidipine and nebivolol from rabbit plasma. The method provided excellent selectivity and linearity with a limit of quantification of 200 ng/ml and 20ug/ml respectively. It has been successfully applied to pharmacokinetic study.

The present study introduced the pharmacokinetic characteristics of cilnidipine and nebivolol tablets administered to rabbit. Besides, the current investigation provides a specific, sensitive, precise, accurate and rapid assay for cilnidipine and nebivolol in human plasma. From the results we conclude that the developed method can be applied in bioequivalence, pharmacokinetic and bioavailability studies of cilnidipine and nebivolol tablets with desired accuracy and precision.

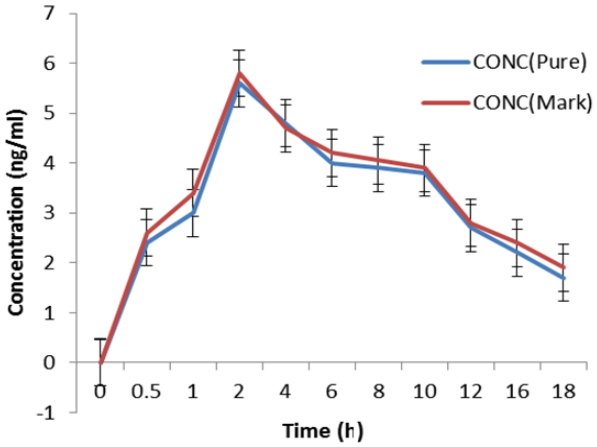

Fig. 9: It shows mean plasma concentrations (mean±SD) of nebivolol versus time profile obtained after a single dose of LN BETA 10 mg+5 mg tablet administered to 6 healthy male rabbits

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AUTHOR CONTRIBUTION

All the author were equally contributed in the research and preparation of manuscript.

CONFLICT OF INTERESTS

Declared none

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