Research Article

Development and Validation of First Order Derivative UV Spectrophotometric Method For Simultaneous Estimation of Nebivolol and Cilnidipine in Pharmaceutical Formulation

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ABSTRACT

A simple, accurate, reliable and reproducible first order derivative method was developed for the simultaneous determination of Nebivolol (NBV) and Cilnidipine (CIL) in pharmaceutical formulation. The linearity was carried out by using the concentration range 4-20 µg/ml for NBV (221.6 nm ZCP of CIL) and 5-25 µg/ml for CIL (249 nm ZCP of NBV). The correlation coefficient of NBV and CIL was found to be 0.999 and 0.998 respectively. At zero crossing point (ZCP) of NBV (249 nm) CIL shows a measurable absorbance, whereas at zero crossing point (ZCP) of CIL (221.6 nm) NBV shows a measurable absorbance value. Precision study shows that %RSD was found to be within range of acceptable limits (< 2%). The % recovery for NBV and CIL was found to be within range of and 98-102%. The percentage assay was found to be 101.8% and 101% for NBV and CIL respectively. The result of analysis has been validated as per ICH Q2 (R1) guideline. This method has applied successfully for determination of NBV and CIL in its pharmaceutical formulation.

Keywords: Nebivolol (NBV), Cilnidipine (CIL), UV Spectrophotometry, First order derivative Spectrophotometry.

INTRODUCTION

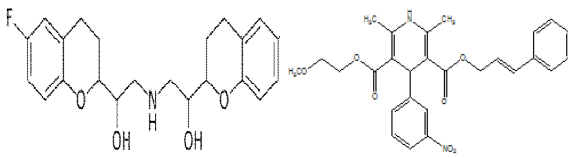
A combined fixed dose formulation containing Nebivolol and Cilnidipine is available as tablet dosage form for treatment of hypertension. Nebivolol (NBV) α,α'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzpyran-2-methanol], is used for treatment of hypertension through vascular endothelial nitric oxide releasing capabilities and β1-antagonist action.[1] Nebivolol is official in IP[2]. Cilnidipine (CIL) 1,4-Dihydro-2,6-dimethyl-4(3nitrophenyl)3,5pyridinedicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester. Cilnidipine (CIL) is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater selectivity for vascular smooth muscle. Cilnidipine is not official in IP, BP and USP.[3,4]

Pharmacological Rationale

A combined fixed dose formulation LN βeta5 containing Nebivolol and Cilnidipine is available as tablet dosage form for treatment of hypertension. Cilnidipine is alone required 20 mg once daily and Nebivolol is alone required maximum dose 40mg/day. The combinations of both the drugs reduce the dose of individual drug. A pharmaceutical composition of Nebivolol 5 mg and Cilnidipine 10 mg provided excellent synergistic effect on lowering blood pressure.

Analytical Rationale

From Literature survey, various methods (UV, HPLC, HPTLC)[5-13] were reported for the analysis of individual drug and in combination with other drug but no method were reported for simultaneous estimation of Nebivolol and Cilnidipine. Hence, the purpose of the present work was to develop and validate Derivative spectrophotometric method for simultaneous estimation of Nebivolol and Cilnidipine in combined dosage form.



(a) Nebivolol (b) Cilnidipine

Figure 1: Chemical structure of Nebivolol (a) and Cilnidipine (b).

MATERIALS AND METHODS

Instrumentation

A UV-visible spectrophotometer, model UV 1800 (Shimadzu) was used to measure absorbance of the resulting solutions using UV-Probe software version 2.31.

A Digital analytical balance (Wensar DA13-220) and ultrasonic sonicator (Equitron) were used in the study.

Reagents and Materials

Pure Nebivolol (NBV) & Cilnidipine (CIL) kindly gifted as a gift sample by Cadila pharmaceuticals, Ahemdabad, India and Niksan pharmaceutical, Ankleshwar, India respectively.

LN βeta 5 Tablet formulation procured from local market. Solvent Methanol (AR Grade) was used in the study. NBV was soluble in Methanol, Acetonitrile and practically

soluble in Water. CIL was soluble in Methanol, Acetonitrile and practically soluble in Water. NBV and CIL were freely soluble in Methanol.

So, Methanol was used as solvent for the preparation of stock and working standard solutions.

Preparation of Solutions

Preparation of Standard Stock Solutions

Nebivolol (100 µg/ml)

Accurately weighed NBV (10 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (100µg/ml).

Cilnidipine (100 µg/ml)

Accurately weighed CIL (10 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (100 µg/ml).

Selection of Analytical Wavelength

The solutions of NBV and CIL were prepared in Methanol by pipette out 1.2 ml and 1.5 ml from the stock solutions (100 µg/ml) at NBV and CIL in respectively in 10ml volumetric flask and diluted up to the mark with Methanol to get concentration 12 µg/ml and 15 µg/ml NBV and CIL respectively.

The solutions were scanned in the wavelength range of 200-400 nm.

The first order derivative of the spectra was processed for the selection of analytical wavelengths such that at the zero crossing of one drug and the other drug showed substantial absorbance.

It was observed that NBV shows ZCP at 249nm and CIL shows ZCP at 221.6 nm. At ZCP of NBV (249 nm), CIL showed a measurable absorbance, whereas at ZCP of CIL (221.6 nm), NBV showed a measurable absorbance.

Hence these wavelengths 221.6 nm and 249 nm were selected as analytical wavelengths for determination of NBV and CIL by first order derivative method (Figure 2).

Method Validation

The above proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedures[14] in order to determine the linearity, Accuracy, Precision and Assay of marketed formulation Limit of detection (LOD) and limit of quantitation (LOQ).

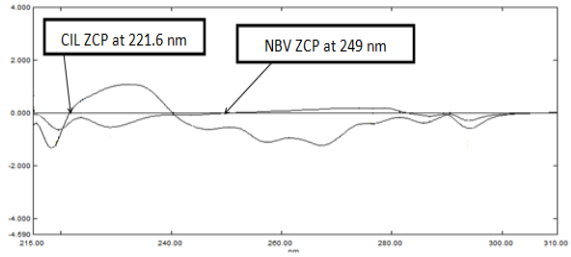


Figure 2: Overlain first order spectra of NBV (12 µg/ml) and CIL (15 µg/ml) in Methanol

Linearity and Range

For Nebivolol

An aliquot of stock solution of NBV (0.4, 0.8, 1.2, 1.6, 2.0 ml) were pipettes out in five different 10ml volumetric flask and further diluted to attain concentration of about 4, 8, 12, 16 and 20 µg/ml respectively.

For Cilnidipine

An aliquot of stock solution of CIL (0.5, 1.0, 1.5, 2.0, 2.5 ml) were pipettes out in five different 10ml volumetric flask and further diluted to attain concentration of about 5, 10, 15, 20 and 25 µg/ml respectively.

The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for NBV and CIL were calculated. R^2 was found to be 0.999 and 0.998 NBV and CIL both the drug respectively.

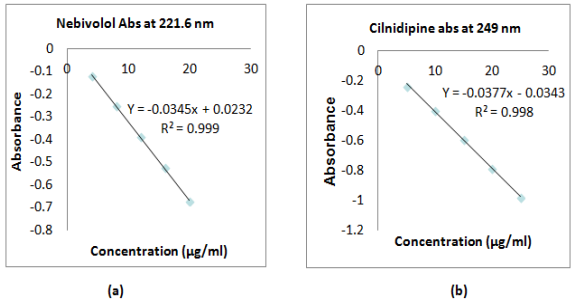


Figure 2: Linearity graph for first order derivative of NBV and CIL

Table 1(a): Calibration Data for NBV and CIL (b) Optical Characteristics

NBV (221.6 nm) (n=3)			CIL (249 nm) (n=3)		
Concentration (µg/ml)	Mean Abs ± SD	%RSD	Concentration (µg/ml)	Mean Abs ± SD	%RSD
4	-0.117 ± 0.001	0.85	5	-0.236 ± 0.002	0.84
8	-0.255 ± 0.002	0.78	10	-0.395 ± 0.003	0.75
12	-0.385 ± 0.003	0.77	15	-0.594 ± 0.003	0.50
16	-0.524 ± 0.003	0.57	20	-0.784 ± 0.004	0.51
20	-0.673 ± 0.004	0.59	25	-0.984 ± 0.002	0.20

Table 1(b)

Parameters	Nebivolol 221nm	Cilnidipine 282 nm
Beer's law limit (µg/ml)	4-20 µg/ml	5-25 µg/ml
Regression equation	Y = -0.0345x + 0.0232	Y = – 0.0377x - 0.0343
Slope (m)	-0.0345	-0.0377
Intercept (c)	0.0232	0.0343
Correlation coefficient (R^2)	0.999	0.998

Table 2: Repeatability, Inter-day and Intra-day precision of Nebivolol and Cilnidipine

Drug	Concentration (µg/ml)	Average ABS ± SD	% RSD
Repeatability (n=6)			
Nebivolol	12	-0.385 ± 0.002	0.51
Cilnidipine	15	-0.593 ± 0.003	0.50
Intra-Day Precision (n=3)			
Nebivolol	8 12 16	-0.254 ± 0.001 -0.385 ± 0.002 -0.524 ± 0.003	0.39 0.51 0.57
Cilnidipine	10 15 20	-0.392 ± 0.002 -0.597 ± 0.002 -0.782 ± 0.003	0.50 0.33 0.38
Inter-Day Precision (n=3)			
Nebivolol	8 12 16	-0.255 ± 0.004 -0.382 ± 0.005 -0.530 ± 0.004	1.56 1.30 0.75
Cilnidipine	10 15 20	-0.394 ±0.003 -0.594 ± 0.004 -0.782 ± 0.005	0.76 0.67 0.63

Table 3: Results of Recovery Studies

Drug	Conc. of std drug	Recovery level (%)	Amount of drug added (µg/ml)	Total Amount of drug (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery ± SD (n=3)
Nebivolol	5	80% 100% 120%	4 5 6	9 10 11	9.12 10.16 11.19	101.3% ± 0.03 101.6% ± 0.03 101.7% ± 0.02
Cilnidipine	10	80% 100% 120%	8 10 12	18 20 22	18.12 20.14 22.18	100.6% ±0.04 100.7% ±0.02 100.8% ±0.03

Table 4: Analysis of Pharmaceutical Formulation

Drug (LN βeta5)	Label claim (mg)	Amount found (mg)	% Drug found ± SD (n=3)
Nebivolol	5	5.09	101.8% ± 0.04
Cilnidipine	10	10.1	101% ± 0.02

Precision Repeatability (n=6)

For the repeatability study, from the working stock solution of both drugs, aliquot of 1.2 ml of Nebivolol was transferred to a separate 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 12 µg/ml and 1.5 ml of Cilnidipine transferred to a separate 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 15 µg/ml. The absorbance of the NBV and CIL was measured at 221.6 nm and 249 nm respectively. The procedure was repeated six times and % RSD was calculated and shown in Table 2.

Intraday Precision (n=3)

From the working stock solution, aliquots of 0.8 ml, 1.2 ml, 1.6 ml of NBV and 1.0 ml, 1.5 ml, 2.0 ml of CIL were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 8, 12 and 16 µg/ml for NBV and 10, 15 and 20µg/ml for CIL. The solutions were analyzed three times on the same day and % RSD was calculated and shown in Table 2.

Interday Precision (n=3)

From the working stock solution, aliquots of 0.8 ml, 1.2 ml, 1.6 ml of NBV and 1.0 ml, 1.5 ml, 2.0ml of CIL were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 8, 12 and 16 µg/ml for NBV and 10, 15 and 20µg/ml for CIL. The solutions were analyzed three times on three different days and % RSD was calculated and shown in Table 2.

Accuracy

The accuracy of the developed method was determined by calculating %recovery at three different levels (80%, 100%, and 120%) in pre analyzed samples 5 mg NBV and 10 mg CIL using standard addition method. The results of recovery studies are reported in table.

The recovery of NBV and CIL are within 98%-102%, assuring that the developed method can estimate the drugs successfully in presence of excipients.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analyzed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified.

The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ were 0.17 µg/ml and 0.52 µg/ml for NBV and 0.17 µg/ml and 0.52 µg/ml for CIL respectively.

$$LOD = 3.3 \times \left(\frac{SD}{Slope}\right)$$

and

$$LOQ = 10 \times \left(\frac{SD}{Slope}\right)$$

Where,

Where,

SD = Standard deviation of the Y - intercepts of the 3 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Assay of Pharmaceutical Formulation

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 5 mg NBV and 10 mg CIL was accurately weighed. The powder was transferred to 100 ml volumetric flask and shaken vigorously with methanol for 15 min and the solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 5µg/ml of NBV and 10 µg/ml CIL respectively. The absorbance of the prepared solutions was measured at 249 nm ZCP of NBV and 221.6 nm ZCP of CIL and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in dosage form was shown in Table 4.

RESULTS AND DISCUSSION

NBV and CIL were freely soluble in Methanol. The first order derivative method is useful for routine analysis of NBV and CIL in pharmaceutical formulation. The zero order and first order spectra for NBV and CIL were recorded and shown in the figure. The zero crossing point was found at the wavelength of 249 nm and 221.6 nm for NBV and CIL respectively (Figure 2).

Linearity and Range

The Beer - Lambert's concentration range was found to be 4-20 µg/ml for NBV and 5-25 µg/ml for CIL at 221.6 nm and 249 nm respectively. The correlation coefficient was found to be 0.999 for NBV and 0.998 for CIL (Table 1) for proposed method.

Precision

Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision (Table 2).

LOD and LOQ

The values of LOD and LOQ were 0.17 µg/ml and 0.52 µg/ml for NBV and 0.17 µg/ml and 0.52 µg/ml for CIL.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (80%, 100%, and 120%). Percentage recovery for NBV and CIL were

found to be in the range of 101.3-101.7 and 100.6-100.8 %. Data indicating recovery studies of NBV and CIL shown in Table 3.

Analysis of the Marketed Formulation

The drug content was found to be 101.8% and 101% for NBV and CIL respectively. This method can be used for routine analysis of NBV and CIL in pharmaceutical dosage form (Table 4).

Table 5: Summary of Validation Parameters for Derivative Spectroscopy Method

S. No.	Parameters	Nebivolol	Cilnidipine
1.	Wavelength	249 nm	221.6 nm
2.	Beer's law limit (µg/ml)	4-20 µg/ml	5-25 µg/ml
3.	Regression equation Y= mx + c	Y= -0.0345x + 0.0232	Y = -0.0377x – 0.0343
4.	Correlation coefficient (R^2)	0.999	0.998
5.	Repeatability (% RSD, n=6)	0.51	0.50
6.	Interday (n=3, %RSD)	0.63 - 0.76	0.75 – 1.56
7.	Intraday (n=3, %RSD)	0.39 - 0.57	0.33 – 0.50
8.	LOD	0.17	0.17
9.	LOQ	0.52	0.52
10.	Accuracy	101.3% - 101.7%	100.6% – 100.8%

CONCLUSION

First order derivative method was developed for simultaneous estimation of NBV and CIL in their combined formulation. NBV was estimated at 221.6 nm and CIL was estimated at 249 nm. Method was validated as per ICH Q2 (R1) found to be accurate, sensitive and precise and economically feasible. Developed methods were successfully applied for estimation of NBV and CIL in pharmaceutical formulation.

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