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RESEARCH ARTICLE

Simultaneous Determination of Nebivolol and Amlodipine in Combined Dosage Form by Derivative Spectrophotometry

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ABSTRACT:

Simple and accurate method to determine Nebivolol and Amlodipine, in tablet dosage form, was developed and validated using Derivative Spectrophotometry. Derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first-order derivative spectra were obtained at N = 1 (scaling factor), $\Delta\lambda$ = 2.0 nm, and determination was performed at 226.5 nm (ZCP of Amlodipine) for Nebivolol and 245 nm (ZCP of Nebivolol) for Amlodipine over the concentration range of 10-70 µg/mL for both drugs with mean recovery of 100.2 ± 1.25 % and 100.1 ± 1.38 % for Nebivolol and Amlodipine, respectively. Method was validated, and the results were compared statistically. They were found to be simple, sensitive, accurate, precise, reproducible and economical. The method was successfully applied for the determination of Nebivolol and Amlodipine in tablet dosage form without any interference from common excipients.

KEYWORDS: Nebivolol, Amlodipine, Derivative spectrophotometry, zero crossing point

INTRODUCTION:

Nebivolol (NEB), α, α1–(imino bis (methylene)) bis (6-fluoro-3, 4-dihydro-2H -1-benzopyran-2-methanol), is an antihypertensive drug and a selective β1 –receptor antagonist without partial agonist activity[1]. Amlodipine (AML), 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate, is a long-acting calcium channel blocker used as an anti-hypertensive in the treatment of angina[2]. Nebivolol is not official in any pharmacoepia. Amlodipine besylate is official in IP and BP. HPLC[3,4] method describe in IP and BP for its estimation. Literature survey reveals various methods like HPLC[5,6], HPTLC[7], LC-MS[8] and Spectrophotometry[9] for determination of NEB in dosage forms as well as biological fluids. Literature survey reveals spectrophotometry[10] HPLC[11,12] Spectrofluorometry[13] Differential-Pulse Voltammetry[14], LC/MS/MS[15] and HPTLC[16] methods for determination of AML in dosage forms as well as in biological fluids.

Till now there is only one reported UV Spectrophotometry method[17] for simultaneous determination of NEB and AML is reported in combined dosage forms. But there is no reported HPTLC method for simultaneous determination of NEB and AML in combined dosage forms and this combination is not official in any pharmacopoeia so no official method is available for estimation of these two drugs in combined dosage form. Hence, the aim was to develop simple, accurate, precise, reproducible and economical HPTLC method for simultaneous determination of NEB and AML in pharmaceutical dosage form.

EXPERIMENTAL:

Reagents and materials:

NEB and AML besylate powders with 99.94 and 99.96 % purity, respectively, suppliedby Torrent Research Centre (Ahmedabad, India), were used as standard. The tablets (5mg NEB and 5 mg AML per tablet) were procured from the local pharmacy. HPLC grade methanol was purchased from Merck Ltd. (Mumbai, India) Methanol (Analytical reagent grade) was from SDfine chemical (Ahmedabad, India); used for preparation of solutions. The tablet dosage forms were same as described in the high performance liquid chromatographic method.

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Apparatus:
A Shimadzu (Columbia, MD) model 1700 double beam UV/Vis. spectrophotometer with spectral width of 2 nm, wavelength accuracy of ± 0.5 nm and a pair of 10 mm matched quartz cells was used. A Sartorius CP224S (Gottingen, Germany) analytical balance, and an ultra sonic cleaner (Frontline FS 4, Mumbai, India) were also used.

Preparation of standard solution:
Accurately weighed NEB (100 mg) and AML besylate (138.6 mg) equivalent to AML (100 mg) were transferred into 2 separate 100 mL volumetric flasks, dissolved in and diluted up to the mark with methanol to obtain standard solutions of NEB (1000 µg/mL) and AML (1000 µg/mL).

Selection of the wavelength for determination of NEB and AML:
Take 3.5 mL of Standard solutions of NEB and AML were transferred into 2 separate 10 mL volumetric flasks and diluted with methanol to obtain solutions of NEB(35 µg/mL) and AML (35 µg/mL). Spectra of these solutions were scanned in the spectrum mode between 200 and 400 nm, with scan speed of 2400 nm/min vs methanol as a blank. Zeroorder spectra of NEB and AML were treated to obtain corresponding first-order derivative spectra with an interpoint distance of 5 nm.

Derivative Conditions:
Using memory channels, the first-order derivative spectra were overlapped. The zero crossing point (ZCP) values of NEB at which the AML showed some derivative response were noted. The wavelength 245 nm was selected for the determination of AML (where the derivative response for NEB was 0). Similarly, 226.5 nm was selected for the determination of NEB (where the derivative response for AML was 0). Characteristic wavelength (ZCPs) for NEB and AML were confirmed by varying the concentration of both drugs.

Calibration curves (Linearity):
Calibration curves were plotted over a concentration range of 10-70 µg/mL for NEB and AML, each. Standard solutions of NEB and AML (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with methanol. The first-derivative absorbance (D1) was measured at 226.5 nm for NEB and 245 nm for AML. The calibration curves were constructed by plotting absorbance versus concentrations and the regression equations were calculated. Each response was average of three determinations.

Preparation of sample solution:
Twenty tablets were weighed and the average weight was calculated. The tablets were powdered and quantity of the powder equivalent to 5 mg NEB and 5 mg AML was transferred to a 50 mL volumetric flask containing methanol (35 mL). The flask was sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was diluted up to the mark with methanol. The solutions were filtered through a Whatman filter paper no. 41. An aliquot (3.5 mL) was transferred to a 10 mL volumetric flask, and the volume adjusted to the mark with methanol. The derivative response was measured at 226.5 and 245 nm for NEB and AML, respectively. The amounts of NEB and AML present in the sample solution were determined by fitting the derivative responses into the regression equation of the respective calibration curves.

RESULTS AND DISCUSSION:

At 245 nm (zero crossing point of NEB), AML showed a first derivative maximum absorbance, whereas at 226.5 nm (zero crossing point of AML), NEB showed a first derivative maximum absorbance. Hence, the wavelengths 226.5 nm and 245 nm were selected for determination of NEB and AML, respectively. The overlaid spectra for NEB and AML are shown in Fig. 1. The overlaid first derivative spectra are shown in Fig. 2. First-derivative spectra give good quantitative determination of both drugs at their respective ZCPs without any interference.

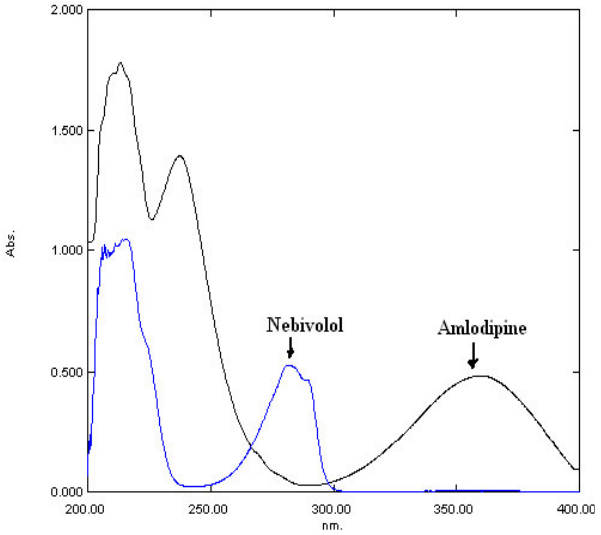

Fig 1 Overlaid zero-order spectra of NEB (35 µg/mL) and AML (35 µg/mL) in Methanol

Validation of the Proposed Method:
Linearity **-** Linear correlation was obtained between absorbance versus concentrations in the range of 10- 70 µg/mL for Derivative Spectrophotometric Method for NEB and AML, respectively. The linearity of the calibration curves was validated by the value of correlation coefficients of regression (Table 1).

Table 1. Regression analysis of calibration curve for NEB and AML for the Proposed Derivative Spectrophotometry

Parameters	Derivative Spectrophotometry method	
	NEB	AML
Linearity range(µg/mL)	10-70	10-70
Slope	0.0016	0.0013
Standard deviation of slope	0.0002	0.0002
Intercept	0.00093	0.0055
Standard deviation of intercept	0.0002	0.0010
Correlation coefficient, *r*	0.9977	0.9957

Table 2. Summary of validation parameters for the proposed Derivative Spectrophotometry

Parameters	Derivative Spectrophotometry	
	NEB	AML
LODa, µg/mL	0.33	0.42
LOQb, µg/mL	1.00	1.26
Accuracy, % (*n* = 3)	99.72-100.28	99.40 -99.89
Repeatability, % RSDc (*n*=6)	0.52	0.49
Precision, % RSD		
Interday (*n* = 6)	0.84-1.64	0.78-1.48
Intraday (*n* = 6)	0.54-1.38	0.62-1.29

aLOD = Limit of detection, bLOQ = Limit of quantification, cRSD = Relative standard deviation, *n* = number of determinations

Table 3. Results of recovery study for NEB and AML by the Derivative Spectrophotometry method (n=3)

Method	Drug	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean % Recovery ±SD[a]*
Derivative Spectrophotometry method	NEB	5	50	99.51 ± 1.79
		5	100	100.7 ± 1.21
		5	150	100.82 ± 1.05
	AML	5	50	99.69 ± 1.37
		5	100	99.49 ± 1.01
		5	150	99.95 ± 1.41

[a]SD = Standard deviation, n = Number of determinations

Table 4. Assay results for the combined dosage form using the proposed HPLC and Derivative Spectrophotometry (*n* = 6)

Parameters	Derivative Spectrophotometry method	
Brand A	NEB ± SD[a]	AML ± SD[a]
	99.80 ± 1.21	99.89 ± 1.75

[a]SD = Standard deviation, *n* = Number of determinations

Table 5. Comparison between results obtained by the Derivative spectrophotometric methods (*n* = 6)

Parameters	Derivative Spectrophotometry		Reported method	
	NEB	AML	NEB	AML
Assay, % ±SD[a]	99.80 ± 1.21	99.89 ± 1.75	99.80 ± 1.21	99.89 ± 1.75
t-value (2.31)[b]	1.78	1.36	-	-

[a]SD = Standard deviation, b Figures in the parentheses represent corresponding to *t*-tabulated value at 95% confidence interval
n = number of determinations

***Accuracy* -** The mean recoveries obtained 99.72 ± 0.15 % and 99.40 ± 0.19 %, for NEB and AML, respectively by Derivative Spectrophotometric Method (Table 3). The values indicate that methods are accurate.

***Method precision* -** The RSD values for NEB and AML was found to be 0.52 and 0.49 for Derivative Spectrophotometric Method (Tables 2). The values of RSD indicate the proposed method is repeatable.

***Intermediate precision* -** The RSD values of interday (0.84-1.64 % and 0.78-1.48 %) and intraday (0.54-1.38 % and 0.62- 1.29 %) variations for NEB and AML, respectively by Derivative Spectrophotometric Method reveal that the proposed method are precise (Table 2).

***LOD and LOQ* -** LOD for NEB and AML were found to be 0.43 µg/mL and 1.09 µg/mL, respectively by Derivative Spectrophotometric Method. LOQ for NEB and AML were found to be 1.3 µg/mL and 3.3 µg/mL, respectively by Derivative Spectrophotometric Method (Table 2).

ASSAY OF THE PHARMACEUTICAL FORMULATION:

The proposed validated method was successfully applied to determine NEB and AML in their combined dosage form (Brand A) without any interference of excipients. The results obtained for NEB and AML were comparable with the corresponding labeled amounts (Table 4).

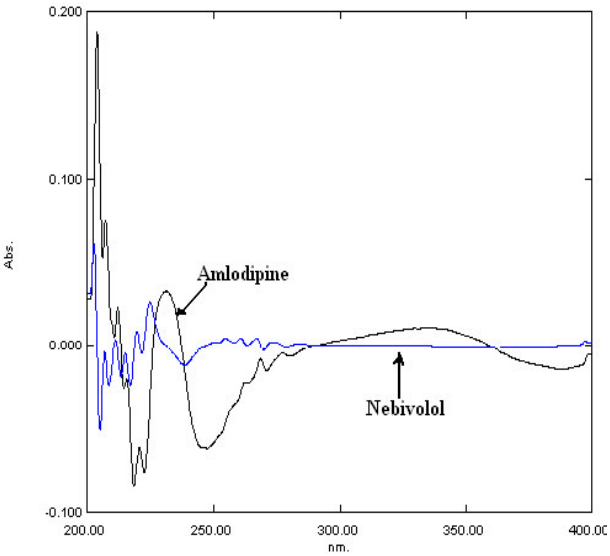

Fig 2 Overlaid first derivative spectra of NEB (35 µg/mL) and AML (35 µg/mL) in methanol

COMPARISON OF THE PROPOSED METHODS:

The assay results for NEB and AML in their combined dosage forms obtained using Derivative Spectrophotometric Method and reported method were compared by applying paired *t*test. The calculated *t* value of NEB (1.78) and AML (1.36) were less than the tabulated *t*value (2.31) at 95%

confidence interval. Statistical comparison of the results obtained by proposed Derivative Spectrophotometric Method and reported method shows good agreement and indicates no significant difference in the content of NEB and AML by the Derivative Spectrophotometric Method and reported method (Table 5).

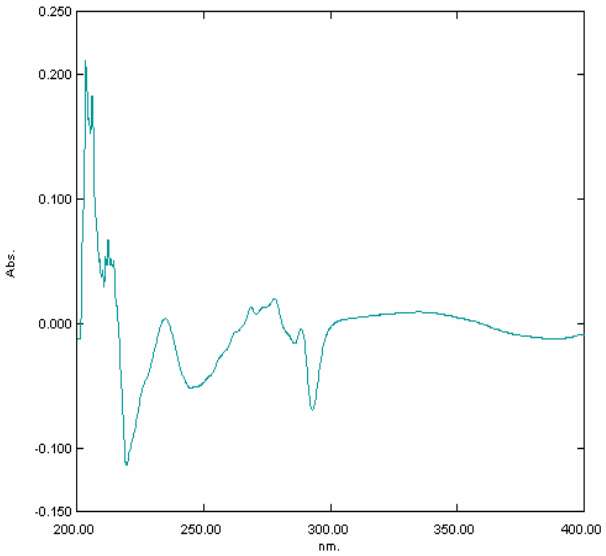

Fig 3 First derivative absorption spectra of sample solution of Nebivolol and Amlodipine in methanol

CONCLUSION:

The result of the analysis of pharmaceutical formulation by the proposed method is reproducible and reliable and is in good agreement with the label claim of the drugs.

The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of NEB and AML. The method can be used for the routine analysis of the NEB and AML in combined dosage form.

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Abbreviations

NEB: Nebivolol
AML: Amlodipine
µg: Microgram
mL: Mililiter
µL: Microliter
LOD: Limit of detection
LOQ: Lmit of quantitation
SD: Standard deviation
RSD: Relative Standard Deviation

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