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SIMULTANEOUS UV-SPECTROPHOTOMETRIC DETERMINATION OF AMLODIPINE BESYLATE AND NEBIVOLOL HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT: Two simple spectrophotometric methods have been developed for simultaneous determination of Amlodipine besylate and Nebivolol hydrochloride in tablet formulation. The first method is Absorbance correction method based on determination of Amlodipine besylate at 365 nm using its absorptivity value and Nebivolol hydrochloride at 280 nm after deduction of absorbance due to Amlodipine besylate. The second method is based on Absorbance ratio in which wavelengths selected were 269 nm, an isoabsorptive point and 280 nm as λ_{max} of Nebivolol hydrochloride. The methods were validated in terms of accuracy, precision, ruggedness and specificity. The methods can be routinely adopted for quality control of these drugs in tablet.
Key words: Amlodipine Besylate, Nebivolol Hydrochloride, UV-Spectrophotometric determination

INTRODUCTION

A combined fixed dose formulation containing Amlodipine besylate (ADB) and Nebivolol hydrochloride (NBH) is available as tablet dosage form for treatment of hypertension and angina pectoris. ADB is chemically known as (4 R, S)-3-ethyl-5-methyl 2-(2-amino-ethoxymethyl)-4-(2-chlorophenyl)-1, 4-dihydroxy-6-methylpyridine-3, 5-dicarboxylate monobenzene sulphonate. B.P describes a reversed phase high performance liquid chromatographic (RP-HPLC)[1] method for the determination of ADB in bulk and pharmaceutical formulations. The literature survey reveals numbers of methods are reported for the quantitative determination of ADB alone or in combination with other anti hypertensive drugs including Spectroscopic and Chromatographic methods[2-6]. Nebivolol hydrochloride[7], chemically, (±)-[2R*[R*[R*(S*)]]]-α,α'[Imino-bis-(methylene)]jh-bis-[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride, a new antihypertensive drug, is a racemate of two enantiomers with two chiral centers. The methods are also reported for estimation of NBH in single dosage form by RP-HPLC[8]. The other methods include estimation of Nebivolol in plasma[9] by HPLC with fluorescence determination and location of hydroxyl functions in hydroxylated metabolites of Nebivolol in different species[10].

However, there is no evidence in literature for simultaneous determination of ADB and NBH. Hence present work describes two spectrophotometric methods for estimation these two drugs simultaneously from tablet dosage form.

MATERIALS AND METHODS

A SHIMADZU model 1700 digital double beam UV-Visible spectrophotometer with 1 cm matched quartz cells was used for absorbance measurements. Pure drug samples of ADB and NBH were kindly gifted by Cadila Healthcare Ltd., Baddi and Hetero Drugs Ltd., Hyderabad respectively. Double distilled water (laboratory made) and dimethyl formamide (DMF) analytical reagent grade were used as solvents in this work. Marketed formulation was procured from commercial source.

PREPARATION OF STANDARD STOCK SOLUTIONS OF ADB AND NBH:

The stock standard solutions containing 1mg/mL of amlodipine and nebivolol were prepared by dissolving 50 mg of amlodipine and nebivolol respectively in sufficient quantity of DMF and diluting up to the mark in a 50 mL volumetric flask with DMF.

ABSORPTION CORRECTION METHOD (METHOD 1):

Stock solutions of ADB and NBH were diluted further with double distilled water to get working concentrations (10µg/mL) of amlodipine and nebivolol for the spectrophotometric study. The diluted solutions were scanned over the wavelength range of 250-400 nm. From the overlain spectra (**Figure-I**), wavelengths 365 λ_{max} of ADB and 280 nm the λ_{max} of NBH were selected for quantitation by proposed method. For studying Beer's law, two series of different concentrations in range of 5-25 µg/mL for amlodipine and nebivolol were prepared from stock solutions. The calibration curves were constructed at 280 and 365 nm respectively. The absorptivities (A1%, 1 cm) of both the drugs at both the selected wavelengths were determined. The quantitative determination of ADB is carried out by using A (1%, 1cm) value at a 365 nm where NBH, interfering substance does not have any absorption and quantitation of NBH is carried out by subtracting absorption due to ADB, interfering drug in the overlapping region of spectrum, on the basis of its absorption ratio at two wavelengths.

ABSORPTION RATIO METHOD (METHOD 2):

The quantitation of ADB and NBH by proposed method was done using the selected wavelengths, 280 nm was taken as λ_{max} for NBH and 269 nm, an isobestic point for estimation of ADB, respectively. Series of different concentrations in range of 5-25 µg/mL for ADB and NBH were prepared from stock solutions. The calibration curves were constructed and regression analysis (**Table I**). was carried out at 280 and 269 nm. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelengths were determined. By using the following equations, one can easily find out the concentration of the individual drug in admixture at the two wavelengths.

For estimation of ADB:

$$c_x = \frac{Q_m - Q_y}{Q_x - Q_y} \frac{A}{ax} \qquad \text{---------- (1)}$$

And for estimation of NBH:

$$c_y = \frac{Q_m - Q_y}{Q_y - Q_x} \frac{A}{ay} \qquad \text{---------- (2)}$$

where,

c_x and c_y are concentrations of ADB and NBH respectively (g/100mL in final solution),

Q_x = the ratio of absorptivity of ADB at 280 and 269 nm.

Q_y = the ratio of absorptivity of NBH at 280 and 269 nm.

Q_m = the ratio of absorbance of mixture at 280 and 269 nm

A = the absorbance of mixture at isoabsorptive point.

ax = the absorptivity value of ADB at isoabsorbtive point

ay =the absorptivity value of NBH at isoabsorptive point.

ASSAY:

An amount equivalent to two tablets (10 mg of amlodipine and 10 mg of nebivolol) was taken into a 10 mL volumetric flask and shaken for about 10 min with 5 mL of DMF, diluted up to the mark with DMF. The contents of the flask were filtered using a Whatman No.41 filter paper. Aliquot portion of the filtrate was further diluted with double distilled water to achieve a concentration of 10µg/mL of amlodipine and nebivolol respectively (On labeled claim basis). The above solution was analyzed for the content of ADB and NBH using the methods described above.

RESULTS AND DISCUSSION

For absorption correction method, the overlain spectra of both the drugs showed the λ_{max} of 280 nm for NBH and 365 nm for ADB where NBH does not show a significant absorption. Hence these wavelengths were selected for estimation of ADB and NBH. Absorbances were determined at both the wavelengths. Both the drugs obeyed linearity in the concentration range of 5-25 µg/mL and the correlation coefficient (r^2) was <1 in both the case. The absorptivity was then calculated and along with absorbance. The content of ADB was determined at 365 nm using its absorptivity value and NBH at 280 nm after subtraction of absorbance due to ADB deduced using absorptivity value at 280 nm.

In absorption ratio method, two wavelengths are selected from overlain spectra out of which one is isobestic point and another is λ_{max} of one of the drugs. The spectra of ADB and NBH when overlaid indicated that the isobestic point was at 269 nm at which estimation of ADB was done and estimation of NBH was done at its λ_{max}, 280 nm. The absorptivity was then

calculated and along with absorbance, these values were submitted in the equations 1 and 2 to obtain concentration of drugs.

Both the methods were successfully used to estimate the amounts ADB and NBH in marketed tablet formulation containing amlodipine 5 mg and nebivolol 5 mg. The results obtained were comparable with the corresponding labeled amounts (**Table II).**

The experiment was repeated three times in a day for intra-day and on three different days for inter-day precision. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at five different levels.

By observing the validation parameters (**Table III)** , accuracy, intra-day and inter-day precision expressed as %RSD, reproducibility (% RSD), specificity, linearity (correlation coefficient, r ^{2}sub <1) and range, both the methods were found to be specific, accurate, precise, repeatable, and reproducible. Hence, both methods can be employed for routine analysis of tablets for assay.

Table I: REGRESSION ANALYSIS OF THE CALIBRATION CURVES

Method	Drug	Wavelength(nm)	Concentration Range (µg/ml)	Intercept	Slope	r^2
1.	ADB	365	5-25	0.0004	0.0162	0.9999
		280	5-25	0.0052	0.0033	0.9999
	NBH	365	5-25	-	-	-
		280	5-25	-0.0017	0.0136	0.9999
2.	ADB	269	5-25	0.0030	0.0080	0.9999
		280	5-25	0.0052	0.0033	0.9992
	NBH	365	5-25	-	-	-
		269	5-25	-0.0008	0.0082	0.9998

Method 1= Absorbance Correction Method and Method 2= Absorbance Ratio Method
r^2= Correlation Coefficient

Table II: ASSAY RESULTS OF AMLODIPINE BESYLATE AND NEBIVOLOL HYDROCHLORIDE IN MARKETED FORMULATION

Tablet	Method 1		Method 2	
	% ADB	%NBH	%ADB	%NBH
Nodon-AM	99.10 ± 0.69	100.03 ± 0.71	99.64 ±1.09	100.19 ± 0.59

Method 1= Absorbance Correction Method and Method 2= Absorbance Ratio Method

Table III: SUMMARY OF VALIDATION PARAMETERS

Parameters	Method 1		Method 2	
	ADB	**NBH**	**ADB**	**NBH**
Linearity Range (µg/ml)	5-25	5-25	5-25	5-25
Correlation coefficient	At 365 nm 0.9999 At 280 nm 0.9999	At 365 nm ---- At 280 nm 0.9999	At 269 nm 0.9992 At 280 nm 0.9999	At 269 nm 0.9998 At 280 nm 0.9999
Precision (R.S.D)				
Ruggedness(%RSD)	0.0179	0.0178	0.0083	0.0200
Intraday (n=3)				
Interday (n=3)	1.7389	1.1635	0.8293	1.1418
Accuracy (%)	0.3828	0.9332	1.1851	0.8084
Reproducibility	100.68	99.10	100.20	99.28
Specifity	Reproducible Specific		Reproducible Specific	

Method 1= Absorbance Correction Method and Method 2= Absorbance Ratio Method
RSD= Relative Standard Deviation

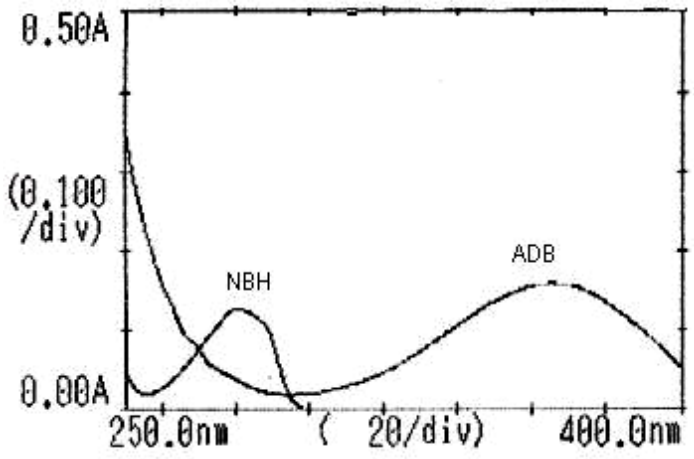

Figure I: Overlain Spectra of ADB and NBH.

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