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Research Article

SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL HYDROCHLORIDE AND AMLODIPINE BESYLATE IN TABLETS

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ABSTRACT

Nebivolol hydrochloride and Amlodipine besylate in combination are available as tablet dosage forms in the ratio of 1: 1. Two simple, sensitive, accurate, and reproducible methods have been developed for simultaneous estimation of both. The proposed methods are based on the partial simultaneous equation method, using methanol as solvent. Nebivolol hydrochloride has absorbance maxima at 281 nm and Amlodipine besylate at 238 and 360 nm. As nebivolol hydrochloride shows zero absorbances at 238 nm and 360 nm. So workable wavelengths selected were 238 nm and 281 nm for a Method I and 281 nm and 360 nm for Method II. Estimation of Amlodipine besylate was done directly from its absorbance at 238 nm and 360 nm for method I and II respectively. While estimation of nebivolol hydrochloride is done by the equations derived. The method is validated statistically. The recovery studies confirmed the accuracy of the proposed methods.

Keywords : Nebivolol hydrochloride, Amlodipine besylate, Spectrophotometry.

INTRODUCTION

Nebivolol hydrochloride is chemically 1-(6-fluoro-chroman-2-yl) – [2-(6-fluoro-chroman-2-yl) 2-hydroxyethylamino]- ethanol hydrochloride, is a highly selective β1 adrenergic antagonist which produces nitric oxide mediated vasodilation[1]. A survey of literature revealed a HPLC-fluorescence method[2], a UV - colorimetric methods[3] and HPLC enantiometric resolution of nebivolol on normal and reversed amylose based chiral phases[4]. Amlodipine besylate is R ,S -2-[(2-aminoethoxy) methyl]- 4 (2-chloroethyl) 3-ethoxy carbonyl – 5 methoxy carbonyl – 6 methyl – 1,4 dihydropyridine benzene sulphonate used in the treatment of hypertension and congestive heart failure[5-7]. The drug is not official in any pharmacopoeia. Literature survey reveals that there are a number of methods for the individual determination of amlodipine like RP-HPLC[8-9], difference spectrophotometry[10], colorimetry[11], HPTLC[12-13], GC[14-15], LC mass spectrophotomety[16], and few spectro-photometric methods[17-21]. There is no suitable method available for the estimation of nebivolol hydrochloride and amlodipine besylate in combined dosage forms.

MATERIALS AND METHODS

Instrument

A GBC Cintra 10 double beam UV-Visible spectrophotometer equipped with 10 mm matched quartz cells was used in the present investigation. A sartorius analytical balance was used.

Chemicals and reagents

All the chemicals used were of analytical grade. Methanol A.R. grade was procured from Loba Chem. Ltd., Mumbai. The commercially available tablets of nebivolol hydrochloride and amlodipine besylate combination, Nodon-Am (CADILA PHARMA)

and Nebicard-SM (TORRENT) were procured from local market. Nebivolol hydrochloride was given by M/s. Torrent Pharmaceuticals Ltd., Ahmedabad and Amlodipine besylate from Ipca lab. Mumbai, as a gift sample. Drugs were used as such without further purification.

Preparation of standard solutions

Stock solutions of nebivolol hydrochloride and amlodipine besylate were prepared by dissolving accurately weighed 100 mg each of standard drugs in 100 ml methanol. Working standard solutions of both were prepared by taking 1 ml of stock solution of nebivolol hydrochloride and amlodipine hydrochloride in 10 ml volumetric flasks and making up the volume with methanol.

Methods of analysis

Overlain spectrum showed the absorption maxima of nebivolol hydrochloride at 281 nm and that of amlodipine besylate at 238 nm and 360 nm (*Figure I*). It was quite astonishing to note that nebivolol hydrochloride does not show any absorbances at 238 nm and 360 nm. So workable wavelengths selected for Method I were 238 nm and 281 nm and for Method II were 281 nm and 360 nm. Both the methods were based on partial simultaneous equation.

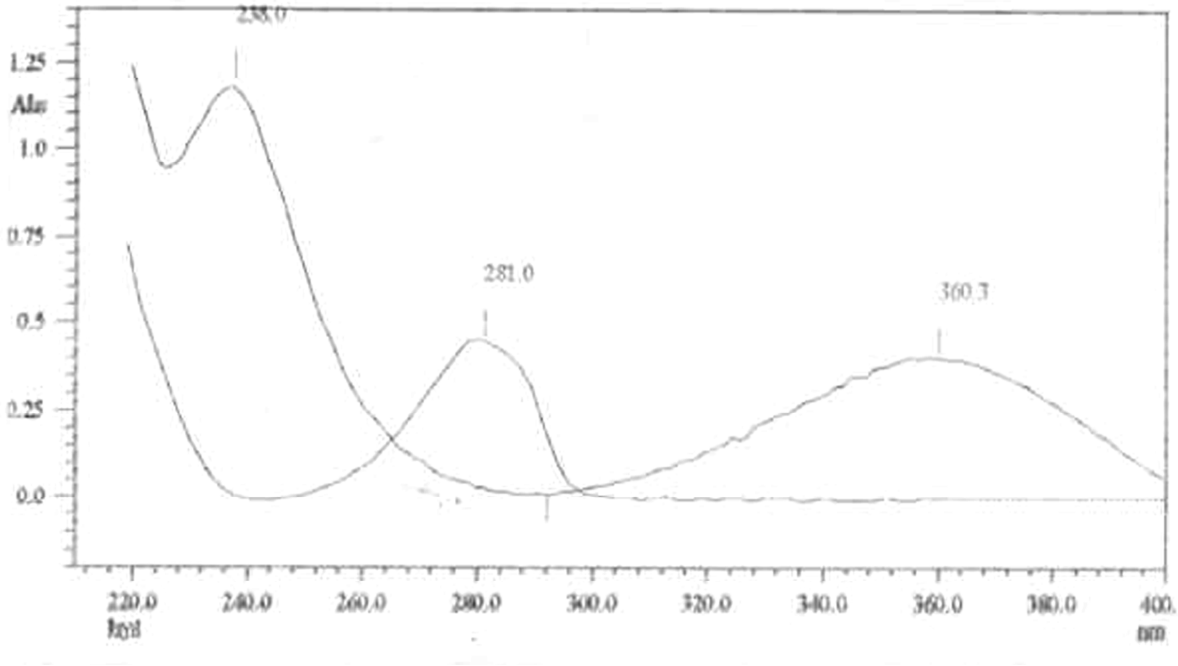

Fig. 1 : Overlain spectrum of nebivolol hydrochloride and amlodipine besylate

The calibration curves for nebivolol hydrochloride and amlodipine besylate were prepared in the concentration range of 10-65 µg/ml and 5-45 µg/ml respectively. Table 1 shows the Optical and Regression characterstics of both the drugs. The values of correlation coefficient suggest the level of precision of the method. The value of molar absorptivity and sandell's sensitivity values show the sensitivity of both the drugs at all the wavelengths. The absorptivity coefficients were determined for both of the drugs at all the wavelengths and following equations were made:

Table 1 : Optical characteristics

Parameters	**At 281 nm**		**At 238 nm**	**At 360 nm**
	Nebivolol hydrochloride	**Amlodipine besylate**	**Amlodipine besylate**	**Amlodipine besylate**
Beer Lambert's law limits (µg/ml)	10 – 65	5 – 45	2 – 20	5 – 45
Molar Absorptivity (1 $mole^{-1}cm^{-1}$)	6.4956×10^4	6.2381×10^2	2.1322×10^4	7.2021×10^3
Sandell's Sensitivity (mg/cm^2/0.001 absorbance unit)	0.0030	0.0515	0.0015	0.0044
Regression equation y=mx+c				
Slope (m)	0.0146	0.001	0.0376	0.0125
Intercept (c)	0.0012	0.0006	0.002	0.004
Correlation coefficient (R^2)	0.9996	0.9924	0.9983	0.9982

Where x = concentration in µg/ml and y = absorbance unit

Table 2 : Compilation of results of statistical analysis of commercial formulations

Tablet Brand	Method	Tablet components	Label claim* (mg/tab)	Amount found* (mg/tab)	SD*	%RSD*	SE*
Nodon Am	Method-I	Nebivolol Hydrochloride	5 mg	4.884 ± 0.0208	0.0260	0.5340	0.0106
		Amlodipine Besylate	5 mg	4.961 ± 0.0171	0.0214	0.4329	0.0087
	Method-II	Nebivolol Hydrochloride	5 mg	4.886 ± 0.0199	0.0249	0.5109	0.0101
		Amlodipine Besylate	5 mg	4.945 ± 0.0264	0.0330	0.6682	0.0134
Nebicard SM	Method-I	Nebivolol Hydrochloride	5 mg	4.774 ± 0.0160	0.0200	0.4193	0.0081
		S-Amlodipine Besylate	2.5 mg	2.441 ± 0.0064	0.0081	0.3318	0.0033
	Method-II	Nebivolol Hydrochloride	5 mg	4.772 ± 0.0201	0.0252	0.52087	0.0102
		S-Amlodipine Besylate	2.5 mg	2.483 ± 0.0125	0.0157	0.6322	0.0064

*Average of six determinations.

In the present case two methods were developed, (based on partial simultaneous equation). The method employs solving of simultaneous equations using Cramer's rule and matrices.

Method-I (Wavelengths selected were 238 nm and 281 nm),The equations developed was

$A_1 = 0.0376\ Cy$, $A_2 = 0.0147\ Cx + 0.0011\ Cy$

Method-II (Wavelengths selected were 281 nm and 360 nm), The equations developed was

$A_2 = 0.0147\ Cx + 0.0011\ Cy$, $A_3 = 0.0127\ Cy$

Using these equations the concentrations of nebivolol hydrochloride and amlodipine besylate were estimated in commercial formulations.

Estimation from tablets

Twenty tablets of each formulation were accurately weighed and average weights was calculated for each formulation.These tablets were ground to a fine powder. An accurately weighed tablet powder equivalent to 50 mg each of nebivolol hydrochloride and amlodipine besylate was taken in case of Nodon Am. While for Nebicard Sm powder equivalent to 50 mg Nebivolol hydrochloride and 25 mg Amlodipine besylate, were taken separately in 100 ml of volumetric flask and extracted quantitatively with (4 × 20 ml) of methanol, insoluble excipients were separated by filteration. Volume was made up with methanol. The solutions so obtained was suitably diluted with methanol to form working standard solution corresponding to 50 μg/ml of each. Aliquots of a definite concentration were taken from working standard solution in six replicates in six 10 ml volumetric flasks. (In Beer Lambert's law limit). The volumes were made and absorbances noted at all the wavelengths. Absorbances obtained were substituted in the equations.

Recovery studies and validation of the method (according to ICH Q2A guidelines)

To study the validation parameters accuracy, reproducibility, reliability and interference, recovery experiment was carried out by standard addition. The recovery of added standard was calculated at different concentration levels. From the total amount of drug found the percentage recovery was calculated. Table 3 shows recovery studies results of nebivolol hydrochloride and amlodipine besylate in synthetic mixture.

Table 3 : Compilation of results of drug recovery study

Tablet Brand	Method	Percentage Recovery ± SD*	
		Nebivolol Hydrochloride	Amlodipine Besylate
Nodon Am	I	99.58 ± 0.2315	99.19 ± 0.1661
	II	99.52 ± 0.2549	98.09 ± 0.2965
Nebicard SM	I	98.98 ± 0.3022	99.95 ± 0.1265
	II	99.07 ± 0.3109	98.01 ± 0.2513

*Average of six determinations.

RESULTS AND DISCUSSION

Drug content in tablet (amount found) was directly found from equations for both the methods. Standard deviations, Coefficient of variation, Standard error of mean, Percentage range of error (Within 95% confidence limits) was calculated (Table 2). The low standard deviation values indicated repeatability,

accuracy and reproducibility of the methods. Reproducibility, reliability and interference was also confirmed by recovery studies. Thus, it can be concluded that the methods developed were simple, accurate, sensitive and precise. Hence, the above methods can be applied successfully in simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in marketed formulations. Statistical analysis and drug recovery data showed that Method-I was more sensitive, accurate and precise.

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