ISSN : 0974-7419 Volume 16 Issue 5

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 16(5) 2016 [220-225]

Simultaneous spectrophotometric estimation of amlodipine and nebivolol in tablet dosage form

Bhushan M.Firake[1]*, sanjay G.Walode[2], sadip D.Firke[3]

[1]Department of Pharmaceutical Analysis, JSPM's Jayawantrao Sawant College of Pharmacy & Research, Handewadi Road, Hadapsar, Pune, Maharashtra, 411028, (INDIA)

[2]Department of Pharmaceutical Analysis, Sinhgad Institute of Pharmaceutical Sciences, Lonavala, Tal. Maval, Dist. Pune, Maharashtra, 410401 (INDIA)

[3]Department of Pharmaceutical Chemistry, SES's, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist. Dhule, Maharashtra, 425 405, (INDIA)

E-mail: bmf.jscopr@gmail.com

ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of amlodipine and nebivolol (in pure and in their pharmaceutical dosage form). The method involves solving of simultaneous equations based on measurement of absorbances at two wavelengths 242.0nm and 286.8nm which are the wavelength maxima of amlodipine and nebivolol, respectively. The method was successfully applied for simultaneous determination of amlodipine and nebivolol in binary mixture.

KEYWORDS

Amlodipine;
Nebivolol;
Antihypertensive agents;
Simultaneous equations;
UV spectrophotometry.

INTRODUCTION

Amlodipine {2-[(2-amino ethoxy) methyl)]-4-(2-chloro phenyl)-3-ethoxycarbonyl-5-methoxy carbonyl - 6- methyl- 1, 4- dihydropyridine} *(Figure 1)* is a calcium channel blocker. It occupies the plasma membrane dihydropyridine receptor and causes competitive blockage of voltage operated slow calcium channel.[1] Nebivolol {1-(6-fluorochroman-2-yl)-2-[[2-(6- fluorochroman-2-yl)-2-hydroxy-ethyl] amino] ethanol} *(Figure 2)* is a competitive and highly selective, third generation β_1-blocker which achieves blood pressure control by modulating nitric oxide (NO) release. Nitric oxide is produced in artery walls and acts to relax vascular smooth muscle cells. It also inhibits platelet aggregation and adhesion and may protect against vascular damage as it inhibits leukocyte activation and vascular smooth muscle cell proliferation.[2] Hence the combination of amlodipine and nebivolol is indicated for the treatment of hypertension and angina pectoris.

Amlodipine and nebivolol are not official in any of the Pharmacopoeia and hence no official meth-

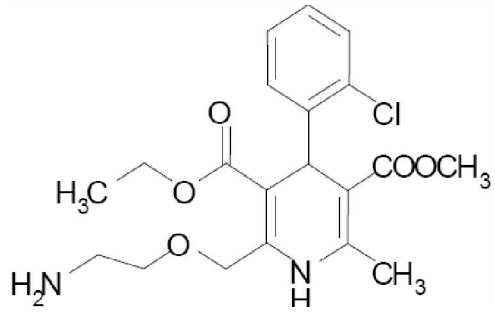

Figure 1 : Chemical structure of amlodipine

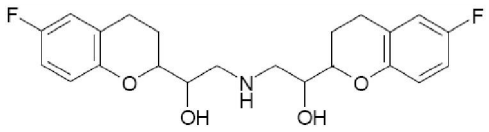

Figure 2 : Chemical structure of nebivolol

ods for their estimation have been reported. For amlodipine, other methods reported in literature are a spectrophotometric method[3], fluorimetric[4], RP-HPLC[5], and HPTLC[6]. While for nebivolol, HPLC[7] and LC-MS[8] methods have been reported.

There is no any method reported for simultaneous estimation of amlodipine and nebivolol in fixed dose combination. The aim of this study was to develop a simple, accurate, economical and reproducible method for simultaneous spectroscopic estimation of amlodipine and nebivolol in two-component tablet formulation.

EXPERIMENTAL

Amlodipine and nebivolol were obtained as gift samples from Torrent Pharmaceuticals Ltd., Indrad and Cadila Pharmaceuticals Ltd., Ahmedabad, respectively. Methanol used was of analytical grade and obtained from Qualigens. A commercial tablet formulation (Nodon AM®) each containing 5mg of amlodipine and 5mg of nebivolol were procured from the local pharmacy. PC based Systronics

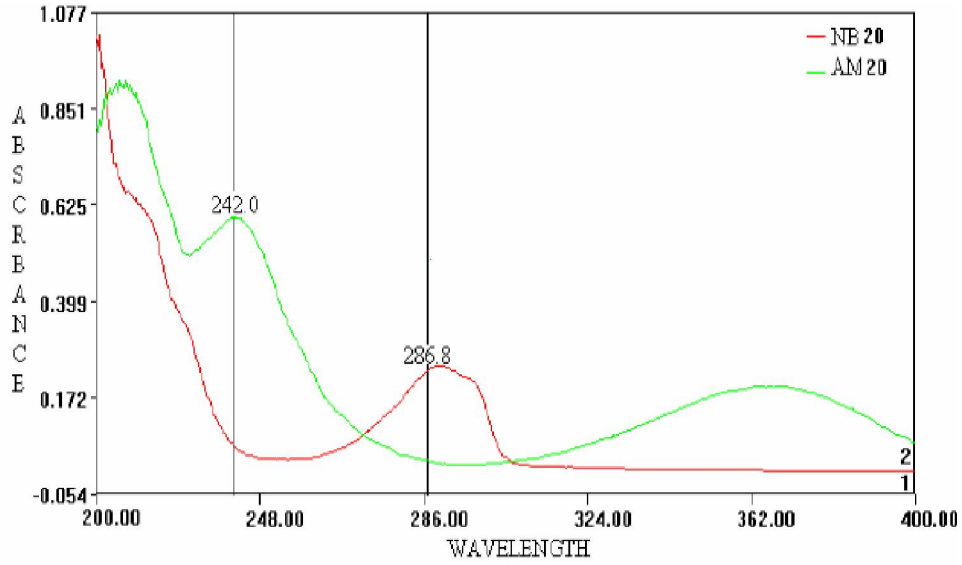

Figure 3 : Overlain spectra of amlodipine and nebivolol in the ratio 1:1

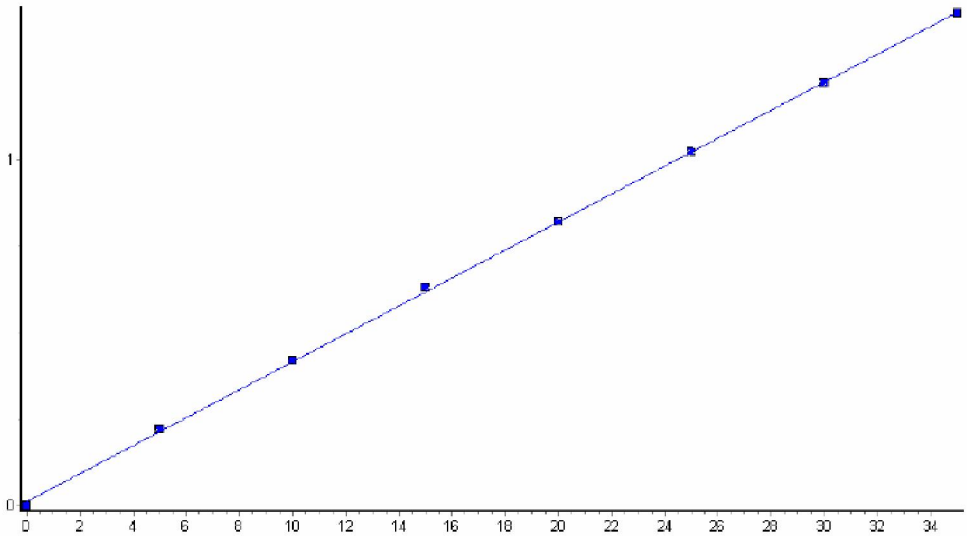

Figure 4 : Calibration curve of amlodipine at 242.0nm.

Full Paper

Double Beam Spectrophotometer 2202 (with 10mm matched quartz cell).

Preparation of standard solutions

Standard stock solution of amlodipine and nebivolol were prepared by separately dissolving 10mg of amlodipine and nebivolol, respectively in 100ml methanol. Stock solutions were diluted separately with methanol so as to get final concentration 20µg/ml of each amlodipine and nebivolol.

Determination of absorption spectra and analytical wavelength

Working standard solutions as prepared above were scanned in the range 200-400nm against methanol as blank to determine wavelength of maximum absorption. The overlain spectrum *(Figure 3)* was recorded using 10mm cell over the range 400nm to 200nm against blank. Wavelengths of maximum absorption (λ_{max}) were found to be 242.0nm and 272.4nm for amlodipine and nebivolol, respectively. Simultaneous equations method was developed for analysis of amlodipine and nebivolol.

Procedure for calibration curve

For each drug, appropriate aliquots were pipetted out from standard stock solution into a series of eight 10ml volumetric flasks. Volume was made up to the mark with methanol to get solutions of concentrations of 0, 5, 10, 15, 20, 25, 30 and 35µg/ml of each. Calibration curve for amlodipine (*Figure 4*) was constructed by plotting absorbance at 242.0nm against its concentration and Calibration curve for nebivolol (*Figure 5*) was constructed by plotting absorbance at 272.4nm against its concentration.

Data for regression analysis is given in *TABLE 1*. By using the quantitative mode of instrument, intercept and slope values were obtained. Slope values for amlodipine and nebivolol were 0.0403 and 0.0061, respectively. Intercept values were 0.013 and 0.0002 for amlodipine and nebivolol, respectively. The correlation coefficients for amlodipine and nebivolol were 0.9998 and 0.9997, respectively.

The absorptivity values for amlodipine and nebivolol at both the wavelengths are presented in *TABLE 2 and 3*, respectively.

The method employed simultaneous equations using Cramer's rule and matrices. A set of two simultaneous equations was framed using the absorptivity coefficient values as given below,

$$\mathbf{A_1 = (476.62 \times C_1) - (23.80 \times C_2)} \quad \mathbf{(1)}$$

$$\mathbf{A_2 = (15.93 \times C_1) - (132.39 \times C_2)} \quad \mathbf{(2)}$$

Where, C_1 and C_2 are concentration (gm/100ml) of amlodipine and nebivolol, respectively in the sample solution. A_1 and A_2 are the absorbances of the sample solution measured at 242.0nm and 286.8nm, respectively. 476.62 and 23.80 are the absorptivities at 242.0nm while 15.93 and 132.39 are the absorptivities at 286.8nm of amlodipine and nebivolol, respectively.

By applying the Cramer's rule and matrices to equation 1 and 2, concentrations C_1 and C_2 can be

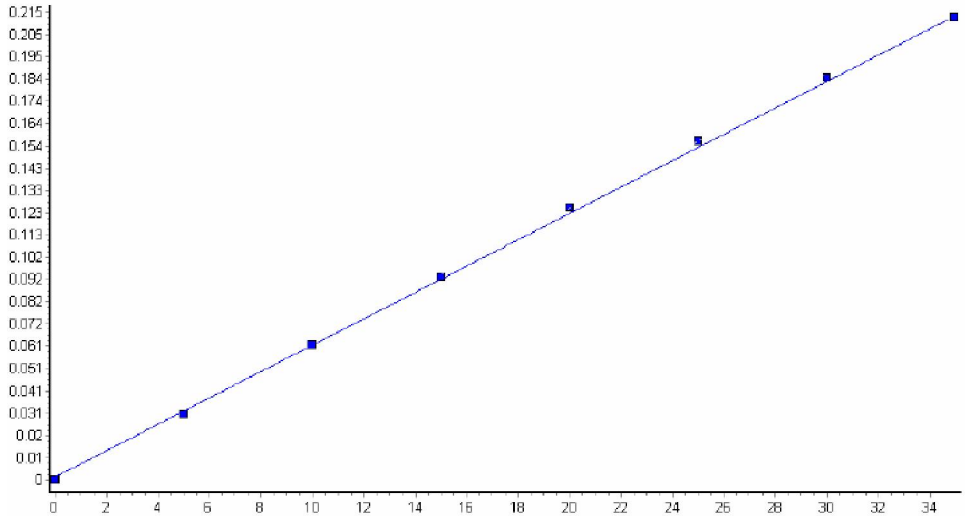

Figure 5 : Calibration curve of nebivolol at 286.8nm.

TABLE 1 : Regression analysis of calibration curves

Parameters	Amlodipine	Nebivolol
Wavelength (nm)	242.0	272.4
Concentration range (µg/ml)	0-35	0-35
Intercept	0.013	0.0002
Slope	0.0403	0.0061
(S.D.)	(0.0004)	(0.0002)
Correlation coefficient(r)	0.9998	0.9997

S.D. = Standard deviation

TABLE 2 : Absorptivity values for amlodipine

Sr. No.	Concentration (g/100 mL)	Absorptivity at 242.0nm	Absorptivity at 286.8nm
1.	0.0010346	477.03	15.92
2.	0.0010104	476.79	15.83
3.	0.0010028	476.63	15.97
4.	0.0009764	475.97	15.86
5.	0.0010252	476.68	16.09
	Mean	476.62	15.93
	± S.D.	0.39	0.10
	C.V.	0.08	0.63

TABLE 3 : Absorptivity values for nebivolol

Sr. No.	Concentration (g/100 mL)	Absorptivity at 242.0nm	Absorptivity at 286.8nm
1.	0.0010102	23.93	133.03
2.	0.0010116	23.79	132.19
3.	0.0010085	23.63	131.88
4.	0.0009772	23.97	132.57
5.	0.0010178	23.68	132.28
	Mean	23.80	132.39
	± S.D.	0.15	0.43
	C.V.	0.63	0.32

obtained as,

$$C_1 = \frac{(A_2 \times 23.80) - (A_1 \times 132.39)}{-62720.58} \quad \textbf{(3)}$$

$$C_2 = \frac{(A_1 \times 15.93) - (A_2 \times 476.62)}{-62720.58} \quad \textbf{(4)}$$

Analysis of laboratory prepared samples

Before analyzing the marketed formulations, the method was validated by analyzing the standard stock solution mixed in appropriate ratios and random samples prepared containing 5, 15, 25µg/ml of amlodipine and 10, 20, 30µg/ml of nebivolol. The results of replicate determinations (n=6) by the proposed method were validated statistically and are shown in *TABLE 4*.

Analysis of marketed formulation

The marketed formulation analyzed was Nodon AM® (manufactured by Cadila Pharmaceuticals Ltd.) containing 5mg amlodipine and 5mg nebivolol using the proposed method.

Twenty tablets were weighed separately and finely powdered. Accurately weighed quantities of tablet powder equivalent to AM (~10mg) were transferred to series of six different 10.0ml volumetric flasks. Methanol (5ml) was taken to it and the flasks were shaken for 10min and volumes were adjusted up to the mark. The solutions were filtered through Whatman Filter Paper No.41. Portions of filtrate were diluted so as to get final concentration of AM

Full Paper

TABLE 4 : Result of analysis of standard samples

Analyte	% Concentration* ± SD	Coefficient of Variance
Amlodipine	100.14 ± 0.26	0.26
Nebivolol	99.25 ± 0.76	0.77

***Average of six determinations. S.D. = Standard Deviation.**

TABLE 5 : Result of analysis of tablet formulation

Formulation	Drug	Label claim (mg)	% Concentration* ± SD	Coefficient of Variance
Nodon AM®	Amlodipine	5	100.22 ± 0.26	0.25
	Nebivolol	5	99.66 ± 0.63	0.63

***. Average of six determinations S.D. = Standard Deviation.**

TABLE 6 : Result of recovery studies

Concentration of added drug to final solution (µg/ml)		% Recovery	
Amlodipine	Nebivolol	Amlodipine	Nebivolol
2	2	101.86	98.80
4	4	99.79	98.20
6	6	101.20	98.12
8	8	102.27	100.20
10	10	101.24	95.20
12	12	100.82	98.20
	Mean	101.19	98.78
	± S.D.	0.86	0.81
	C.V.	0.84	0.82

20µg/ml and NB 20µg/ml. Then the absorbances of these resultant solutions were measured at 242.0nm, 286.8nm as A_1 and A_2, respectively and concentrations of the two drugs in sample were determined using equations (3) and (4). Statistical data of the results obtained after replicate determinations (n=6) are shown in *TABLE 5*.

Recovery studies

To study the accuracy, reproducibility and precision of the proposed method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample. Results of recovery studies were found to be satisfactory and are presented in *TABLE 6*.

RESULTS AND DISCUSSION

From the Overlay Spectra of Amlodipine and Nebivolol *(Figure 3)*, two wavelengths were selected for the estimation of both drugs by Simultaneous Equation Method. A λ_{max} of Amlodipine at 242.0nm and a λ_{max} of Nebivolol at 286.8nm were selected.

The absorbances of both the drugs were found to be satisfactorily at selected wavelengths.

The relationship between concentration and absorbance for individual drugs was studied *(Figure 4 and 5)*. A linear relationship was observed for both the drugs individually over the concentration range 5-35µg/ml (Amlodipine) at 242.0 nm and 5-35µg/ml (Nebivolol) at 286.8nm.

The absorptivity values for both the drugs were determined at the selected wavelengths *(TABLE 2 and 3)*.

On the basis of above studies, Simultaneous UV-Spectrophotometric Method i.e. Simultaneous Estimation Method was evolved and applied to laboratory mixture of both drugs. The results were quite satisfactory *(TABLE 4)* and hence the method was extended for the estimation of drugs in pharmaceutical preparation *(TABLE 5)*.

The values of coefficient of variation were satisfactorily low and recovery was close to 100% for both the drugs *(TABLE 6)*.

The magnitude of analytical background response

was recorded by calculation of limit of detection (LOD) and limit of quantitation (LOQ). An appropriate number of blank samples (9 determinations) were scanned in the selected range and the standard deviations of these responses were calculated. LOD and LOQ were found to be 0.2 and 0.7µg/ml for amlodipine and 0.3 and 1.2µg/ml for nebivolol. The results indicated that the method was sensitive.

ACKNOWLEDGEMENTS

The authors are thankful to Torrent Pharmaceuticals Ltd., Indrad and Cadila Pharmaceuticals Ltd., Ahmedabad for providing standard samples of drugs and also the TVES College of Pharmacy, Faizpur, KYDSCT College of Pharmacy, Sakegaon and JSPM's Jayawantrao Sawant College of Pharmacy & Research, Hadapsar, Pune for providing facilities to carry out work.

REFERENCES

[1] A.R.Gennaro; Eds., In, Remington: The science and practice of pharmacy, 19th Edition, Mack publishing company, **2**, 963 **(1995)**.

[2] R.A.Lakshmana, M.Bharani, R.K.Raja, G.G.Sankar, Nebivolol; A cardioselective β_1-adrenergic blocker, The Antiseptic, **103(1)**, 13 **(2006)**.

[3] N.Rahman, H.M.Nasrul; Spectrophotometric determination of amlodipine besylate in drug formulations, J.Pharm.Biomed.Anal., **31(2)**, 381 **(2003)**.

[4] Y.E.Mohamed, M.E.Nazlaa, A.M.Bahia, G.M.Nashwa; Simultaneous fluorimetric determination of amidarone, Amlodipine and propafenone, Bull.Fac.Pharm.(Cairo Univ.), **36(1)**, 1 **(1998)**.

[5] S.S.Zarapkar, N.S.Kanyawar; Simultaneous estimation of amlodipine and Losartan potassium in pharmaceutical dosage by reverse phase high performance liquid chromatography, Indian Drugs, **34(6)**, 350 **(1997)**.

[6] A.P.Argekar, S.G.Powar; Development of HPTLC method for simultaneous estimation of atenolol and amlodipine in tablet dosage form, J.Pharm.Biomed.Anal., **21**, 1137 **(2000)**.

[7] Y.Hassan, Aboul-Enein, I.J.Ali; Studies on the effect of alcohols on the chiral discrimination mechanisms of amylose stationary phase on the enantioseparation of **nebivolol** by HPLC, Biochem.Biophy.Mtds., **48(2)**, 175 **(2001)**.

[8] N.V.S.Ramakrishna, K.N.Vishwottam, M.Koteshwara, S.Manoj, M.Santosh, D.P.Varma; Rapid quantification of **nebivolol** in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry, J.Pharm.Biomed.Anal., **39(5)**, 1006 **(2005)**.