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Research Article

ABSORBANCE CORRECTION METHOD FOR SIMULTANEOUS DETERMINATION OF NEBIVOLOL AND AMLODIPINE BESYLATE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT
The manuscript describes validated absorbance correction method for the estimation of nebivolol and amlodipine besylate in combined dosage form. Absorbance correction method was based on property of additivity of absorbances. The two wavelengths on amlodipine besylate curve were found out where it showed same absorbance, which were 262 and 332.5 nm. At 332.5 nm, amlodipine besylate showed some absorbance while nebivolol showed zero absorbance. Both the drugs gave absorbance at 262 nm. The method involved solving of an equation based on measurement of absorbance at two wavelengths 262 and 332.5 nm. The determinations were made at 262 for nebivolol and amlodipine besylate and 332.5 nm for amlodipine besylate over the concentration range of 10-70 µg/ml for both nebivolol and amlodipine besylate with mean recovery of 99.7 ± 0.15 and 99.4 ± 0.18 % for nebivolol and amlodipine besylate, respectively by absorbance correction method. This method was found to be simple, sensitive, accurate, precise, reproducible, and economical and can be applicable for the simultaneous determination of nebivolol and amlodipine besylate in combined dosage form.
Key words: Nebivolol, Amlodipine besylate, Absorbance correction method, Validation, Combined dosage form

INTRODUCTION

Nebivolol (NEBI) is an antihypertensive drug and chemically is, α,α[1]–(imino bis (methylene)) bis (6-fluoro-3, 4-dihydro-2 H -1-benzopyran-2-methanol), which is a selective β_1 -receptor antagonist without partial agonist activity[1]. Literature survey reveals HPLC[2-3], HPTLC[4], LC-MS[5] and spectrophotometry[6] methods for estimation of NEBI in single dosage form. Amlodipine besylate (AMLO) is a long-acting calcium channel blocker used as an anti-hypertensive in the treatment of angina and chemically is (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5 pyridinedicarboxylate benzenesulfonate; 2-[(2-Aminoethoxy) methyl]-4-(2-Chloro- phenyl)-3-Ethoxycarbonyl-5-Methoxycarbonyl-6-Methyl-1, 4-Dihydropyridine[1]. It is official in IP. IP describe HPLC[7] method for its estimation Literature survey also reveals spectrophotometry[8], HPLC[9], spectrofluorimetry[10], differential-pulse voltammetry[11], LC[12], LC/MS/MS[13] and HPTLC[14] methods for estimation of AMLO in pharmaceutical formulations and in biological fluids. This combination is not official in any pharmacopoeia hence no official method is available for estimation of these two drugs in combined dosage form. Literature survey reveals HPTLC[15-16] and spectrophotometric[17] method for simultaneous estimation of NEBI and AMLO in combined dosage form. The present manuscript describes alternative simple, sensitive, accurate, precise, reproducible, and economical absorbance correction method for simultaneous estimation of NEBI and AMLO in combined dosage form.

MATERIAL AND METHODS

Apparatus

Shimadzu (UV-1700) double beam UV-Visible spectrophotometer attached with computer operated software UV probe with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Sartorius CP224S analytical balance (Gottingen, Germany), ultra sonic cleaner (Frontline FS 4, Mumbai, India) and corning volumetric flasks were used during the study.

Reagents and Materials

Pharmaceutical grade of NEBI and AMLO was kindly supplied as a gift samples from Torrent Pharmaceutical Ltd, Gujarat (India) with 99.97% purity. The pharmaceutical formulations containing 5 mg NEBI and 5 mg AMLO of brand A and brand B were procured

from the local pharmacy. Methanol (AR grade) were purchased from Finar Chemicals Ltd, Ahmedabad India, and nylon 0.45 µm – 47 mm membranes filter (Gelman Laboratory, Mumbai, India) were used in the study.

Methodology

Absorbance spectrum of pure AMLO was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 332.5 nm (wavelength λ_1, the wavelength of minimum absorbance for AMLO) was noted from spectrum. Then the cursor function was moved along with peak curve until the absorbance equal to that of absorbance at 332.5 nm was found. The wavelength obtain corresponding to this absorbance value was 262 nm (λ_2). The absorbance of various dilutions of AMLO in methanol was measured at 332.5 nm. Absorbance spectrum of pure NEBI was also scanned in the spectrum basic mode. NEBI showed some absorbance value at 262.0 nm (λ_2) while it dose not show any absorbance value at 332.5 nm. The absorbance value at 332.5 nm is due to AMLO only in the combined mixture of both drugs. Wavelength λ_1 (332.5 nm) was selected for the measurement of AMLO.

Preparation of NEBI and AMLO standard stock solutions

A mixed stock solution of NEBI ((100 µg/ml) and AMLO (100 µg/ml) was prepared by accurately weighing NEBI (100 mg) and AMLO (100 mg), dissolving in methanol and diluted to 100 ml with methanol in the same volumetric flask.

Preparation of sample solution

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 5 mg of NEBI and 10 mg of AMLO was transferred to a 50 ml volumetric flask. The content was mixed with methanol (30 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a nylon 0.45 µm membrane filter. The volume was adjusted up to the mark with methanol. An aliquot of this solution (3.5 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with methanol.

Method Validation

The method was validated according to the ICH guidelines[18].

Calibration curve (linearity)

Calibration curves were plotted over a concentration range of 10-70 µg/ml for NEBI and AMLO. Accurately measured mixed standard working solutions of NEBI and AMLO (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol and absorbances were measured at 332.5 nm and 262 nm for both the drugs. The calibration curves were constructed by plotting absorbance at 332.5 nm versus concentrations for AMLO and absorbance difference (A_{262} - $A_{332.5}$) versus concentration for NEBI.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of NEBI and AMLO by the standard addition method. Known amounts of standard solutions of NEBI and AMLO (15, 22.5, 30 µg/ml for both drugs) were added to prequantified sample solutions of tablet dosage form. The amounts of NEBI and AMLO were estimated by applying obtained values (n = 6) to the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instruments was checked by repeatedly injecting (n = 6) standard solutions NEBI and AMLO (40 µg/ml) without changing the parameter for the absorbance correction method.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solutions of NEBI and AMLO (20, 40, and 60 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines[18].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and S = Slope of calibration curve.

Analysis NEBI and AMLO in combined dosage forms

Pharmaceutical formulation of NEBI and AMLO was purchased from local pharmacy. The responses of formulations were measured at 332.5 nm and 262 nm for AMLO and NEBI, respectively by absorbance correction method as described above. The amounts of NEBI and AMLO present in sample solution were determined by fitting the responses into the regression equation for NEBI and AMLO in the method.

RESULTS AND DISCUSSION

Absorbance correction method

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of

an interfering component, two specific wavelengths are chosen.

1. First wavelength λ_1 at which minimum absorbance of AMLO was observed and there was no interference of NEBI at this wavelength (332.5 nm).
2. Second wavelength λ_2 was the wavelengths at which the absorbance of AMLO was same as at λ_1, and also NEBI was also give some absorbance at this wavelength (262.0 nm). .

In this proposed method the absorbance of NEBI alone in a mixture of NEBI and AMLO was determined using dual wavelength data processing program. To remove the interference of AMLO to the absorbance at 262.0 nm (λ_2), the wavelength of minimum absorbance for AMLO, another wavelength 332.5 nm (λ_1) was found out at which the absorbance of NEBI was zero. This was confirmed by measuring the absorbance of various dilution of AMLO in methanol at 262.0 nm and 332.5 nm. The absorbance at these two wavelengths was found to be equal. These two selected wavelengths were employed to determine the concentration of NEBI from the mixture of NEBI and AMLO (Figure 1). The difference in absorbance at these two wavelengths ($A_{262.0} - A_{332.5}$) cancels out the contribution of absorbance of AMLO in mixture.

Validation data of the proposed methods

Linearity - Linear correlation was obtained between absorbance and concentration of NEBI and AMLO in the range of 10-70 µg/ml. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression (Table 1).

Accuracy - The recovery experiments were carried out by the standard addition method. The mean recovery obtained was 99.7 ± 0.15 % and 99.4 ± 0.19 % for NEBI and AMLO, respectively (Table 1). The high values indicate that the method is accurate.

Method precision - The % RSD values for NEBI and AMLO were found to be 1.13 and 0.49 (Table 1). The low values of RSD indicate the proposed method is repeatable.

Intermediate precision - The low RSD values of interday (0.23-1.13 % and 0.73-1.84 %) and intraday (0.53-2.24 % and 0.23-1.99%) variations for NEBI and AMLO, respectively reveal that the proposed method is precise (Table 1).

LOD and LOQ - LOD for NEBI and AMLO were found to be 0.43 µg/ml and 1.09 µg/ml, respectively. LOQ for NEBI and AMLO were found to be 1.3 µg/ml and 3.3 µg/ml, respectively (Table 1). These data show that the method is sensitive for the determination of NEBI and AMLO.

Assay of the pharmaceutical formulation

The proposed validated methods were successfully applied to determine NEBI and AMLO in their combined dosage form (brand A and B). The results obtained for NEBI and AMLO were comparable with the corresponding labelled amounts (Table 2).

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of NEBI and AMLO. The methods can be routinty used for the analysis of the NEBI and AMLO in combined dosage form.

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REFERENCES

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: 2006. p. 83 and 1112.
2. Sahoo MK, Giri RK, Barik CS, Kanungo SK, Ravi kumar BV. RP-HPLC method for the estimation of nebivolol in tablet dosage form. E – J Chem 2009; 6(3):915-919
3. Patel LJ, Suhagia BN, Shah PB. RP-HPLC and HPTLC methods for the estimation of nebivolol hydrochloride in tablet dosage form. Indian J Pharm Sci 2007;69:594-596.
4. Ramakrishna NVS, Vishwottam KN, Koteshwara M, Manoj S, Santosh M, Varma DP. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal 2005;39:1006–1013.
5. Kamila MM, Mondal N, Ghosh LK, Gupta BK. A validated UV spectrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation. Pharmazie 2007;62(7):486-487.
6. Dhandapani B, Suresh K, Kumar J, Dharuman M, Kavitha P. HPTLC method development and validation for the estimation of nebivolol hydrochloride. Pharma info net 2008.
7. Indian Pharmacopoeia, 6th ed, Vol. 2, New Delhi: Controller of Publication. Govt of India; 2007. p. 806 and 1758.
8. Nafisur R, Syed NH. UV-spectrophotometric method for the estimation of amlodipine besylate in combined dosage form. Analytical Sciences 2000; 16(12):1353.
9. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Devlopment and validation of bioanalytical method for the estimation of amlodipine by HPLC method. Farmaco2005;60(9):789-792 .
10. Hanaa M, Niveen AM, Ashraf MMA. Validated spectrofluorometric method for estimation of amlodipine besylate in pharmaceutical formulation. Spectrochim Acta A 2008;70(3):564-570.

11. Altiokka G, Dogrukol-Ak D, Tunçel M, Aboul-Enein HY. A validated differential-pulse voltammetry method for estimation of amlodipine besylate in pharmaceutical formulation. Arch Pharm 2000;335(2):104-108.
12. Malesuik MD, Cardoso SG, Bajerski L, Lanzanova FA. Determination of amlodipine in pharmaceutical dosage forms by liquid chromatography and ultraviolet spectrophotometry. J AOAC Int 2006;89(2):359-64.
13. Taeko Y, Mitsuko T, Kazuhiko I. Development and validation of bioanalytical method for the estimation of amlodipine by LC-MS-MS method. J Mass Spectrometry 1998;31(8):879 – 884.
14. Gowri N, Vaidhyalingam V, Shantha A. Development and validatioin of high performance thin layer chromatography for the simultaneous estimation of Amlodipine besylate (AB) and Benazepril hydrochloride (BH) in tablets. Indian Drugs 2003;40(11):645-648.
15. Dhandapani B, Anjaneyulu N, Venkateshwarlu Y, Harun Rasheed, S. HPTLC method development and validation for the simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in tablet dosage form. J Pharm Res 2010;3(2):332-334.
16. Dangi M, Chaudhari D, Sinker M, Racha V, Mrinalini.C. Stability indicating HPTLC method for estimation of nebivolol hydrochloride and amlodipine besylate in combination. Eurasian J Anal Chem 2010;5(2):161-169.
17. Mishra P, Shah K, Gupta A. Spectrophotometric methods for simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in tablets. International J Pharm Pharm Sci 2009;1(2):55-61.
18. International Conference on Harmonisation (ICH), Topic Q2R1, Validation of Analytical Procedures: Text and Methodology:2005.

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED ABSORBANCE CORRECTION METHOD

Parameters	Absorbance correction method	
	NEBI	AMLO
Concentration range (µg/ml)	10-70	10-70
Slope	0.0061	0.0029
Intercept	0.005467	0.002867
Correlation coefficient (r^2)	0.9952	0.9969
LOD[a] (µg/ml)	0.43	1.3
LOQ[b] (µg/ml)	1.31	3.31
Accuracy (% recovery, n = 6)	99.7 ± 0.154	99.36 ± 0.186
Repeatability (% RSD[c], *n* = 6)	1.13	0.49
Precision (%RSD)		
Interday (*n* = 6)	0.23-1.13	0.73-1.84
Intraday (*n* = 6)	0.53-2.24	0.23-1.99

[a]LOD = limit of detection [b]LOQ = limit of quantification [c]% RSD = percent relative standard deviation

TABLE 2: ASSAY RESULTS FOR THE TABLET DOSAGE FORM

Tablets	Absorbance correction method	
	NEBI± S. D.[a] (n^b = 6)	AMLO ± S. D.[a] (n^b = 6)
Brand A	99.80 ± 1.21	99.89 ± 1.75
Brand B	99.70 ± 0.72	98.50 ± 1.00

[a]S. D. = standard deviation
[b]n = number of determinations

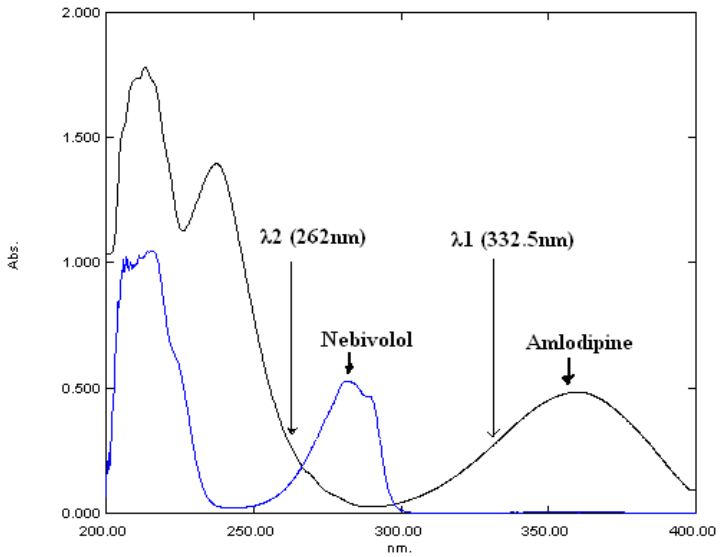

Figure 1: Overlain spectra of standard NEBI and AMLO in methanol

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