

SIMULTANEOUS UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ATENOLOL AND AMLODIPINE BESYLATE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

Two simple, rapid, accurate, precise, cost effective, and reproducible UV spectroscopic methods have been developed for the simultaneous estimation of atenolol and amlodipine besylate in bulk and combined tablet dosage form. The first method is based upon the simultaneous equation and second upon the determination of Q value. Atenolol and amlodipine have absorption maxima at 224.4 and 238.2 nm respectively. Beer's law obeyed in concentration range of 2-24 µg/ml and 2-34 µg/ml for ATN and AMN respectively. The method of Q analysis is based on measurement of absorptivity at 224.4 nm and at iso-absorptive point 232.2 nm. The recovery studies from tablet are indicative of accuracy of method and are found in between 99.87-101.43 % at three different levels of standard additions. Precision studies showed satisfactory results. A novel approach to use 0.02% SLS as solvent is proved to be beneficial with respect to cost, stability and avoidance of organic solvent.

Key Words: Atenolol, Amlodipine Besylate, UV Spectroscopy, Simultaneous equation, Q analysis.

INTRODUCTION

Atenolol (ATN) is chemically 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide, is beta-blocker seem to be equally effective as an antihypertensive, anti-anginal and anti-arrhythmic drug. It is widely used cardiovascular drug in combination with Amlodipine.¹

Amlodipine Besylate (AMN) is chemically 3-ethyl-5-methyl-(4RS)-2-[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate, is calcium channel blocker used as potent coronary and peripheral vasodilator and in bradycardia.¹

Literature survey reveals that various analytical methods have been reported for the assay of atenolol and amlodipine besylate in pure form and in pharmaceutical formulations. Non aqueous titration method is specified in Indian Pharmacopoeia for the assay of atenolol. While British Pharmacopoeia described liquid chromatography method for the assay of amlodipine besylate. Other

methods such as derivative spectroscopy^{2,3}, simultaneous spectroscopic estimation^{4,5}, HPLC⁶⁻¹¹, RP-HPLC^{12,13}, Colourimetry, gas chromatography, difference spectroscopy¹⁴, HP-TLC¹⁵ were reported for the estimation of atenolol and amlodipine in individual formulations and combined dosage forms.

An attempt was made to develop simple, accurate, precise, reproducible, economic and organic solvent free method for simultaneous estimation of both these drugs in combined dosage form.

MATERIAL AND METHODS

Spectrophotometric studies were carried out using Shimadzu UV-Visible spectrophotometer, model-1700 (Japan). Pure samples of amlodipine besylate and atenolol were obtained from Shreya Life sciences Pvt. Ltd. Aurangabad and Lupin Research Park, Pune (M.S.) respectively. The marketed combination of atenolol and amlodipine that is Amlopress AT 50 tablet (Cipla Ltd.). Sodium lauryl sulphate (SLS) was purchased from LOBA Chemie Pvt.Ltd.

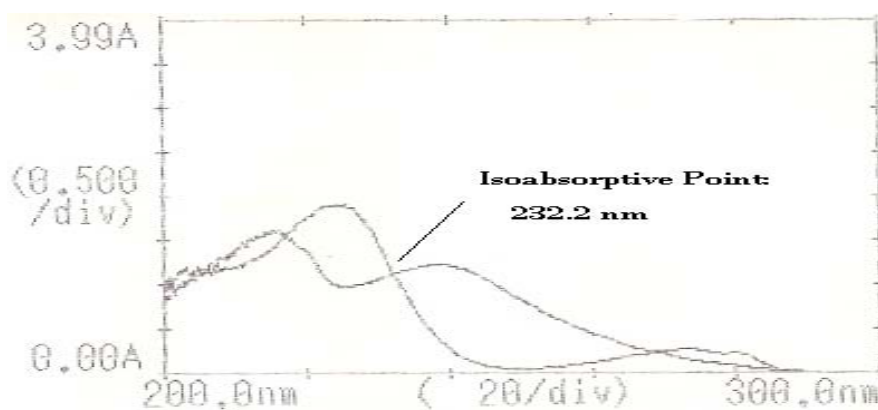


Fig. 1: Overlain spectra of ATN and AMN in 0.02% SLS solution

Selection of common solvent

The selection of common solvent was made after assessing the solubility of both the drugs in different solvents. Both the drugs were found to be practically insoluble in the water. Use of organic solvents such as methanol or ethanol was avoided by using 0.02% SLS solution. SLS solution was made in double distilled water.

Preparation of standard drug solution

Standard stock solutions containing atenolol (ATN) and amlodipine besylate (AMN) were prepared individually by dissolving 10 mg of ATN and quantity of AMN separately in 0.02 % SLS solution. It was then sonicated for 10 min and final volume of both the solutions were made up to 100 ml with 0.02% SLS solution to get stock

solutions containing 100µg/ml each of ATN and AMN in two different 100 ml volumetric flasks.

Assessment of absorption maxima

The dilutions of the stock solutions were prepared by using 0.02 % SLS solution to get the concentrations of 40µg/ml of each drug separately. The two solutions were scanned separately in the range of 200-400 nm to determine respective wavelength of maximum absorption. ATN and AMN showed absorbance maxima at 224.4 nm (λ_1) and 238.2 nm (λ_2) respectively. The overlain spectra showed isosorptive point at 232.2 nm (fig.1).

Method I (Simultaneous estimation method)

The two equations were constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of individual absorbances of ATN and AMN.

$$\text{At } \lambda_1, \quad A_1 = a_{11}bcx + a_{12}bcy \dots (1)$$

$$\text{At } \lambda_2, \quad A_2 = a_{21}bcx + a_{22}bcy \dots (2)$$

Where, A_1 and A_2 are absorbances of mixture at 224.4 nm and 238.2 nm respectively.

Rearrange and substitute the above equations,

$$cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \dots (3)$$

$$\text{For ATN,} \quad c_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{a_1}{A}$$

$$\text{For AMN,} \quad c_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{a_2}{A}$$

Where,

$$Q_0 = \frac{\text{Absorbance of sample at 224.4 nm}}{\text{Absorbance of sample at 232.2 nm}}$$

Application of the proposed method for the determination of ATN and AMN in tablet formulation

Twenty tablets of marketed formulation containing ATN 50 mg and AMN 5 mg were finely powdered. The powder equivalent to 10 mg ATN is weighed and the required amount of pure AMN was added to bring the concentration of AMN in linearity range. The ratio of ATN: AMN as 1:1 was achieved. The weighed quantity was dissolved in 100 ml of 0.02% SLS solution and sonicated and final volume made to 200 ml. The resultant stock solution contains 100 µg/ml ATN and 100 µg/ml AMN. Appropriate aliquots of the stock solution were withdrawn and serial dilutions were performed.

The prepared stock solutions of both the drugs were further diluted separately with 0.02% SLS solution to get the serial dilutions 2-14 µg/ml concentrations. ATN and AMN showed absorbance maxima at 224.4 nm (λ_1) and 238.2 nm (λ_2) respectively. The absorbances were measured at the selected wavelength and absorptivities (A_1 1%, 1cm) for both the drugs at both wavelengths were determined.

Preliminary calculations and assumptions

1. The absorptivities of ATN at λ_1 and λ_2 , ax_1 and ax_2 respectively.
2. The absorptivities of AMN at λ_1 and λ_2 , ay_1 and ay_2 respectively.
3. The absorbances of diluted sample at λ_1 and λ_2 , A_1 and A_2 respectively.
4. Let C_x and C_y be the concentrations of ATN and AMN respectively indiluted sample.

$$cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \dots (4)$$

Method II (Absorbance ratio/ Q value Method)

The overlain spectrum of the two candidate drugs was obtained. The overlain spectrum shown isosorptive point at 232.2 nm. The two wavelengths were selected one as 232.2 nm (Isosorptive point) and other as 224.4 nm (wavelength of maximum absorption of AMN). The serial dilutions were prepared and absorbances were measured and absorptivities for the both the drugs at selected wavelengths were also calculated.

The Q value is used for the estimation of concentrations of drugs in sample solutions. The following formulas are used in the method.

$$Q_1 = \frac{\text{Absorptivity of ATN at 224.4 nm}}{\text{Absorptivity of ATN at 232.2 nm}}$$

$$Q_2 = \frac{\text{Absorptivity of AMN at 224.4 nm}}{\text{Absorptivity of AMN at 232.2 nm}}$$

A = Absorbance of sample at isosorptive point, a_1 and a_2 = Absorptivities of ATN and AMN respectively at isosorptive point.

In Method I, the concentration of both ATN and AMN were determined by measuring the absorbance of the sample at 224.4 nm and 238.2 nm. Values were substituted in the respective formula to obtain concentrations (Table 2).

In Method II, the concentration of both ATN and AMN were determined by measuring absorbance of the sample at 224.4 nm and 232.2 nm and values were substituted in respective formula to obtain concentrations (Table 2).

Table 1: Optical characteristics and other parameters

Parameter	Method I		Method II	
	ATN	AMN	ATN	AMN
Beer's Law Limit (µg/ml)	2-24	2-34	2-36	2-36
Correlation Coefficient (r^2)	0.9987	0.9992	0.9996	0.9988
Molar Absorptivity (lit/mol/cm)	45326.67	3006.54	2699.78	2824.34
Slope	0.9985	0.9987	0.9972	0.9983
Intercept	0.0009	0.0011	0.0029	0.0084

Table 2: Result of Analysis of samples

Analyte	Method I		Method II	
	ATN	AMN	ATN	AMN
% Conc. Estimated* (Mean ± R.S.D.)	100.34 ± 0.32	99.34 ± 0.26	99.16 ± 0.27	100.22 ± 0.35

*Average of three determinations; R.S.D. = Relative standard deviation

Validation of method

The method was validated according to ICH Q2B guidelines for validation of analytical procedure in order to determine sensitivity, linearity, precision, and accuracy.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of standard solution of ATN and AMN. For simultaneous equation method and Q analysis, the Beer's-Lambert's concentration range was found to be 2-24 µg/ml and 2-34 µg/ml for ATN and AMN respectively (Table 2).

Accuracy

To ascertain accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for ATN and AMN by both the methods was found in the range 99.87 % to 101.43 % (Table 3).

Precision

Precision was studied to find out intra and inter-day variations in the test method of ATN and AMN. Calibration curves prepared in medium were run in triplicate in the same day and for three consecutive days. % Relative standard deviation (RSD) were calculated which should be less than 2% (Table 4).

Table 3: Result of analysis of tablet samples

Method	Drug	Label Claim	% Label Claim±RSD	% Recovery (Mean±RSD)
I	ATN	50	101.32±0.564	99.87±0.43
	AMN	5	104.89±2.97	100.32±0.12
II	ATN	50	103.21±1.35	101.43±0.29
	AMN	5	101.87±1.67	99.91±0.16

*Average of three determinations; R.S.D. = Relative standard deviation

Table 4: Determination of Precision

Day	Method I		Method II	
	% Label Claim estimated (Mean±% R.S.D.)			
Intraday	ATN	AMN	ATN	AMN
	99.21±0.61	99.67±0.42	99.88±0.56	99.75±0.22
Interday	99.20±0.39	99.70±0.76	99.90±0.33	99.74±0.91

*Average of three determinations; R.S.D. = Relative standard deviation

RESULTS AND DISCUSSION

The two methods selected for multi-component analysis were given the satisfactory results. A novel approach to use 0.02% SLS as solvent proven beneficial with many respects such as reduction in cost, no use of organic solvent and stable pharmaceutical solvent for analysis. The spectra of ATN and AMN exhibit λ_{max} of 224.4 nm and 238.2 nm respectively. Additionally one isosorptive point was observed at 232.2 nm. These wavelengths were selected for simultaneous estimation and Q analysis of ATN and AMN and are assumed to be sensitive wavelengths. Standard calibration curves for ATN and AMN were linear with correlation coefficient of 0.9972-0.9987 at all selected wavelengths. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of standard additions, recovery in the range of 99.87-101.43 % justifies the accuracy of method.

CONCLUSION

The developed methods were found to be simple, sensitive, accurate, precise, cost effective, reproducible, and can be used for routine quality control analysis in tablet dosage form.

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