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Simultaneous estimation of amlodipine besylate and atenolol in combined dosage form by Vierodt's method using U.V. spectroscopy

P. Y. Pawar*, B. Y. Mane, Sumit M. Auti and Vipul V. Trivedi

Dept. of Pharmaceutical Chemistry, P.D.V.V.P.F' s College of Pharmacy, Ahmednagar, Maharashatra

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ABSTRACT

To develop the new method for simultaneous estimation of Atenolol and Amlodipine Besylate. Spectroscopic studies were carried out using double beam U.V.Spectrophotometer model JASCO.The marketed combination of Atenolol and Amlodipine Besylate that is Primol-AT 10 TAB Madley pharma and 0.1N HCL used as solvent. Then spectra of AML and ATN exhibit λmax of 239nm nand 228nm resepectively.Additionaly one isoisoprtive point was observed at 233nm this wavelength were selected for simultaneous estimation of AML and ATN and standard Calibration curves for AML and ATN were linear with correlation coefficient 0.996 and 0.993 at all selected wavelengths. This method was found to be applicable over a range of 4-24ug/ml for AMLand ATN.This method can be used as alternative for rapid and routine determination of bulk sample and tablets.

Keywords: Amlodipine Besylate; Atenolol; UV Spectrophotometry; Vierodt's method.

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 antiarrhymthmic drug. It is widely used cardiovascular drug in combination with Amlodipine. Amlodipine besylate (AMN) is chemically 3-ethyl-5-methyl-(4RS)-2-[(2-aminoethoxy) methyl] -4-(2-chlorophenyl)-6-methyl-1,dihydropyridine-3, 5-dicarboxylate benzene sulphonate, is calcium channel blocker used as potent coronary and peripheral vasodilator and in Bradycardia.

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 aq ueous 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, simultaneous spectroscopic estimation, HPLC, RP-HPLC ,Colourimetry, Gas Chromatography, Difference Spectroscopy, HP-TLC were reported for the estimation of Atenolol and Amlodipine Besylate in individual formulations and combined dosage forms. An attempt was made to develop simple, accurate, precise, reproducible, economic method, organic solvent free method for simultaneous estimation of both these drugs in combined dosage form.

MATERIALS AND METHODS

INSTRUMENT -
JASCO Double beam UV-VIS Spectrophotometer with spectral band width of 1.8 nm,wavelength accuracy of ±2 nm and matched quartz cells of 10 mm optical path length was used for all spectral and absorbance measurements.

MATERIALS-

Double beam UV-VIS Spectrophotometer (JASCO)with spectral band width of 1.8nm ,wavelength accuracy of ± 2nm and matched qurtz cell of 10mm optical path length was used for all specteral and Absorbance measurement.Atenolol and Amlodipine Besylate were obtained as a gift sample from Alembic Pharma and marketed formulation from Spectrophotoscopic studies were carried out using Double beam UV Visible spectrophotometer, model-JASCO. 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 Primol- AT 10 tablet (Medleys Pharma) and 0.1N HCL used as solvent.

METHOD

A) Preparation of stock solution-

1. Pure drug-

Accurately weighed 10mg pure drug of AML and ATE were dissolved in the 0.1N HCL in a two different 10ml volumetric flask and sonicated for 10min.Then from this solution pipette out 1ml from each in separate 10ml volumetric flask and named as stock solution B. 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 dilution were prepared such as 4ug,8ug,12ug,16ug,20ug,24ug per 1ml as per its linerity.

2. **Marketed formulation-**

The Average weight of 10 tablets was taken and it was found to be 0.168gm,which contain claim of 5mg and 50mg AML and ATE respectively. As per label claim ,Accurately weighted of drug was taken and in a 10 ml of volumetric flask contain 10ml 0.1N HCL.The solution was sonicated for 20min and was filtered through Whatman filter paper no.40 .From this solution sufficint aliqut was pipette out into 10ml volumetric flask and from these serial dilution was prepared.The absosbance values of these solution were measured at 239nm and 228nm respectively

1. Assessment of absorption maxima

The two solutions were scanned separately in the range of 200-400 nm to determine respective wavelength of maximum absorption. AML and ATN showed absorbance maxima at 239 nm (λ_1) and 228 nm (λ_2) respectively.

Simultaneous estimation method

AML and ATN showed absorbance maxima at 239 nm (λ_1) and 228nm (λ_2) respectively. The absorbances were measured at the selected wavelength and absorptivities (A 1%, 1cm) for both the drugs at both wavelengths were determined.

Preliminary calculations and assumptions

1. The absorptivities of AML at λ_1 and λ_2, ax_1 and ax_2 respectively.
2. The absorptivities of ATN 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 Cx and Cy be the concentrations of AML and ATN respectively in diluted sample.

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 AML and ATN.

At λ_1, $A_1=ax_1bcx + ay_1bcy$…… (1)
At λ_2, $A_2=ax_2bcx + ay_2bcy$…… (2)

Where, A_1 and A_2 are absorbances of mixture at239nm and 228 nm respectively.

Validation

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

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%). Percent recovery for Amlodipine and Atenolol by this method and was found in the range of 99.20- 100.75%

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Amlodipine and Atenolol (figures 1 and 2). For simultaneous equation method the Beer- Lambert's concentration range was found to be 4-20 µg/ml for Amlodipine and 4-24 µg/ml for Atenolol.

RESULTS AND DISCUSSION

Analytical data

A linear correlation was found between absorbances at λ_1 and λ_2 max and concentrations of AML and ATE. The optical characteristics such as Beer's law limits, molar absorptivity . Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table No.3 . The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and x concentration in µg/ml. The limit of detection and quantification calculated according to ICH guidelines and reveals a very high sensitivity.

The proposed methods are simple, rapid and precise and do not suffer from any interference due to excipients of tablet. The proposed spectrophotometric methods were found to be linear in the range of 4- 20µg/ml at 239nm in with correlation coefficients (r^2) 0.996 while in 4-24 µg/ml at 228nm with correlation coefficients (r^2) for atenolol were found to be 0.993. The methods were validated in terms of accuracy, precision and repeatability .The accuracy of the method was determined by performing recovery studies by standard addition of method in which pre analyzed samples were taken and standard drug was added at three different levels. Values of recovery greater than 98.0% indicate that proposed method is accurate for the analysis of the drug.

Table 1:Analysis of tablet formulation

Drug	Drug Label claim (Mg/ tab.)	Amount found (mg) %	% Drug found ±SD	Standard Error
Amlodipine	5	4.96	99.3067±0.707	0.4761
Atenolol	50	49.52	100.49±0.504	0.1123

Table 2:Recovery study of Amlodipine and Atenolol

Drug	Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery ± SD
Amlodipine	80	4	4..03	100.75±0.03055
	100	5	4.96	99.2±0.3055
	120	6	5.97	99.5±0.2516
Atenolol	80	8	7.98	99.75±0.03
	100	10	9.92	99.2±0.2
	120	12	11.94	99.5±0.2081

Table 3: Optical characteristics data and validation parameters

Parameters	For Amlodipine Values	For Atenolol values
Absorption maxima (λ max)	239 nm	228 nm
Beer's law limit (µg/ml)	4-20	4-24
Regression equation	Y=0.051x-0.022	Y=0.033x-0.031
Intercept (a)	0.022	0.031
Slope (b)	0.051	0.033
Correlation coefficient (r^2)	0.996	0.993
Molar absorptivity	7.38	26.63
A(1%,1cm)	0.2708	0.7501
Accuracy (%Recovery ± SD)	99.30±1.1618	100.49±0.270
Precision		
Intraday*(Analyst 1)	99.31±0.35	99.75±0.2
Interday*(Analyst 2)	100.86±0.86	98.66±0.81
LOQ (µg/ml)	0.005	0.082
LOD (µg/ml)	0.165	0.249

The precision of the proposed method was estimated in terms of inter day precision and intra day precision where in the method was repeated on three different days and repeated for three different time periods in the same day respectively. SD less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of the drug.

The selectivity of the method was checked by monitoring a standard solution of in Amlodipine and Atenolol presence of excipients at the same concentration level as used in tablet using the method described in the procedure for calibration curve in pharmaceutical tablets. The excipients did not show any effect on the estimation of Amlodipine and Atenolol. This method for the routine analysis of Amlodipine and Atenolol in pharmaceutical preparations.

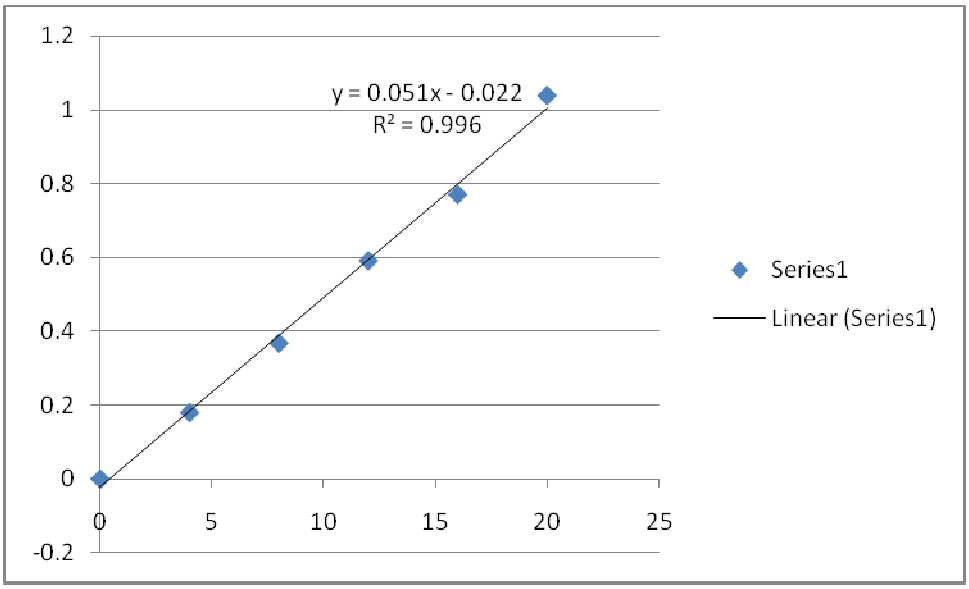

Figure 1: Linearity curve for Amlodipine

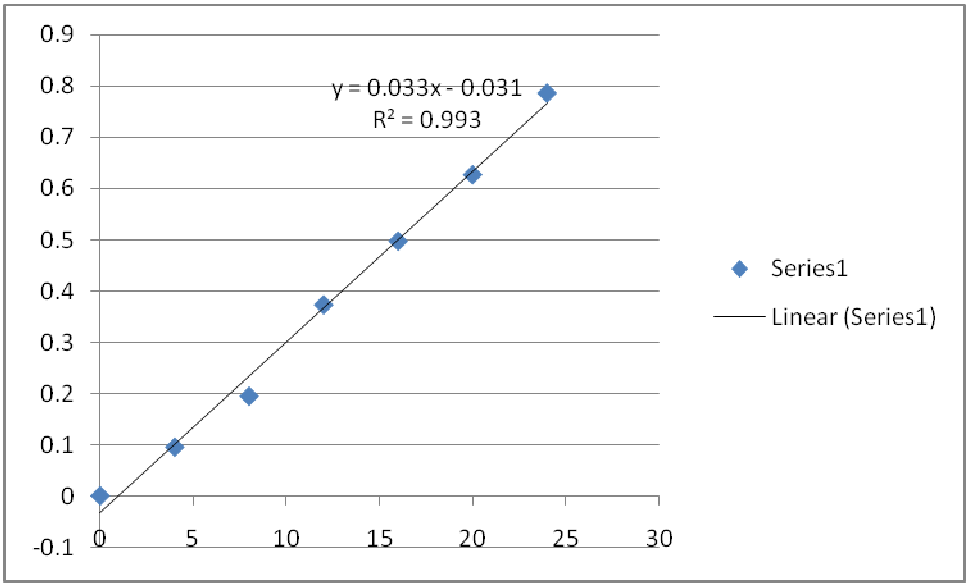

Figure 2: Linearity curve for Atenolol

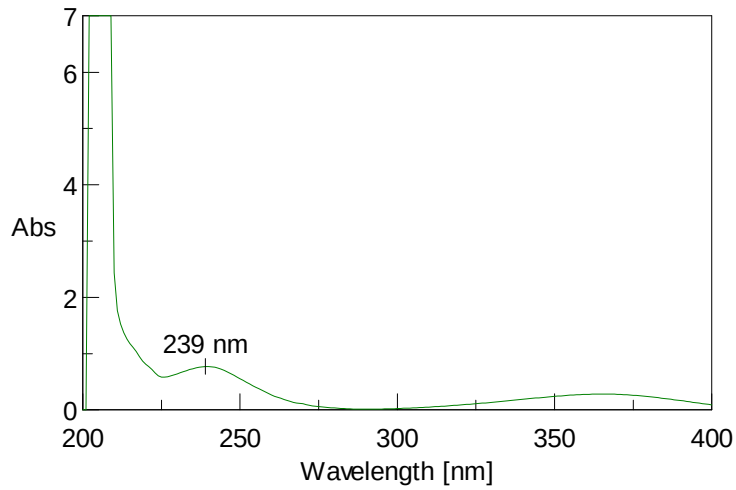

Fig3-The Graph Of Amlodipine API

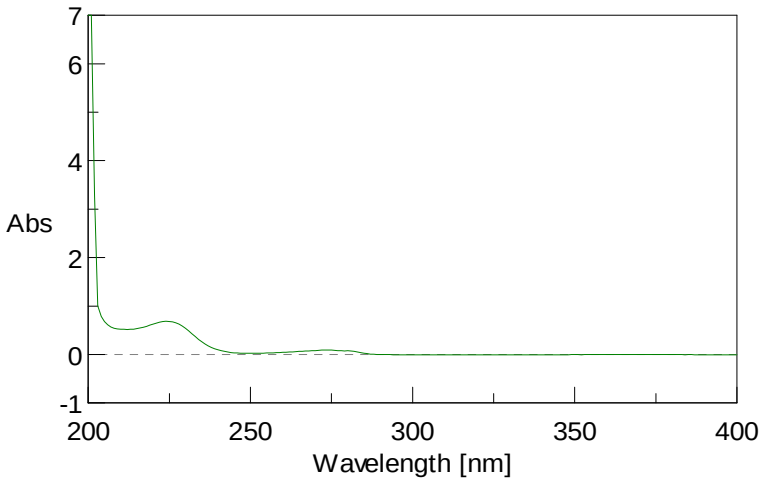

Fig4-The Graph Of Atenolol API

CONCLUSION

The method for the determination of Amlodipine Besylate and Atenolol have been developed and validated. The Method is applicable over a range of 4-20µg/ml for AML and 4-24 µg/ml for ATE. The developed method was 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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