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QUANTITATIVE ESTIMATION AND VALIDATION OF ATENOLOL AND AMLODIPINE BESYLATE BY ABSORBANCE RATIO (Q) METHOD.

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ABSTRACT

The simple, rapid, accurate, precise, cost effective, and reproducible UV spectroscopic method have been developed for the simultaneous estimation of atenolol and amlodipine besylate in bulk and combined tablet dosage form. Atenolol and amlodipine have absorption maxima at 224 and 238.2 nm respectively. Beer's law obeyed in concentration range of 2-24 µg/ml and 2-34 µg/ml for Atenolol (ATN) and Amlodipin (AMN) respectively. The method of Q analysis is based on measurement of absorptivity at 224 nm and at isoabsorptive point 232.2 nm. The recovery studies from tablet are indicative of accuracy of method and are found in between 99.05-101.16% at three different levels of standard additions. Precision studies showed satisfactory results. A novel approach to use 0.1N HCL as solvent is proved to be beneficial with respect to cost, stability and avoidance of organic solvent.

KEYWORDS: Atenolol, Amlodipine Besylate, 0.1 N HCL, Q 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 anti- arrhythmic drug. It is widely used cardiovascular drug in combination with Amlodipine.[1] Beta blockers are also called beta-adrenergic blocking agents. This means that atenolol blocks the action of the stress hormone epinephrine, which is also known as adrenaline. Epinephrine increases the body's heart rate, raises blood pressure, and affects the body's immune system response.

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. Atenolol and amlodipine with other combination such as Q-analysis method[1], H-Point standard addition method.Area under curve and Absorbance ratio method Simultaneous equation method, simple spectroscopic technique.

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.

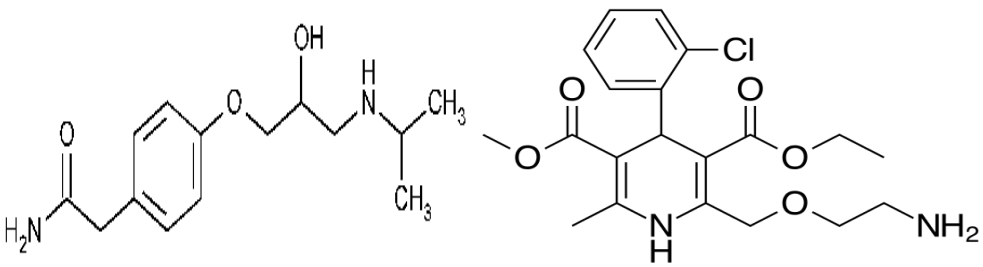

Fig: (a) Structure of Atenolol **(b) Structure of Amlodipine**

MATERIAL AND METHODS

Spectrophotoscopic studies were carried out using Shimadzu UV- Visible spectrophotometer, model-1700 (Japan). Pure samples of amlodipine besylate and atenolol were obtained from Micro labs. Ltd. Banglore and Cipla Ltd, Mumbai (M.S.) respectively. The marketed combination of atenolol and amlodipine that is Amlopress AT 50 tablet (Cipla Ltd.).

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 0.1 N HCL Solution as a common solvent for atenolol and amlodipine.

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 0.1 N HCL Solution. It was then sonicated for 10 min and final volume of both the solutions were made up to 100 ml with 0.1 N HCL Solution to get stock solutions containing 100µg/ml each of ATN and AMN in two different 100 ml volumetric flask.

Assessment of absorption maxima

The dilutions of the stock solutions were prepared by using 0.1 N HCL 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 (λ1) and 238.2 nm (λ2) respectively. The overlain spectra showed isoisorptive point at 232.2 nm (fig.1).

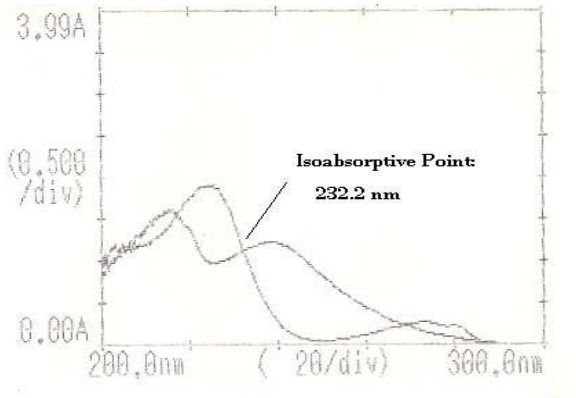

Fig. 1: Overlain spectra of ATN and AMN in 0.1 N HCL

Method (Absorbance ratio/ Q value Method)

The overlain spectrum of the two candidate drugs were obtained. The overlain spectrum shows isoabsorptive point at 232.2 nm. The two wavelengths were selected one as 232.2 nm (Isoisorptive point) and other as 224 nm (wavelength of maximum absorption of ATN). The serial dilutions were prepared and absorbances were measured and absorptivities for the both the drugs at selected wavelengths were also calculated.

Assay of Tablet Formulation

Twenty tablets of Amlopress were weighed and finely powdered and tablet powder Equivalent to 10 mg of both Atenolol and amlodipine is weighed and put in a 100 ml volumetric flask. The flask was sonicated for 15 min and volume was made up to the mark with 0.1 N HCL. 1ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with water, and 1ml of above solution is added to 10ml volumetric flask and made upto the mark with water, finally the solution is filtered by using syringe filter to obtain 10µg/ml of Atenolol and 10 µg/ml of amlodipine. The absorbance of the solution was measured under UV spectrophotometer. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation was calculated in the Table 1. The Q value is used for the estimation of concentrations of drugs in sample solutions. The following formulas are used in the method,

$$Q1 = \frac{\text{Absorbtivity of ATN at 224.4 nm}}{\text{Absorbtivity of ATN at 232.2 nm}}$$

$$Q1 = \frac{\text{Absorbtivity of AMN at 224.4 nm}}{\text{Absorbtivity of AMN at 232.2 nm}}$$

The concentration of both ATN and AMN were determined by measuring absorbance of the sample at 224 nm and 232.2 nm and values were substituted in respective formula to obtain concentrations.

Table: 1 Assay of formulation by Absorption Ratio method

Formulation	Drug	Label Claim	Drug	Amount Found ± SD	% Recovery	%RSD
Amlopress	Atenolol	50	Atenolol	50.12±1.35	101.43	0.29
	Amlodipine	5	Amlodipine	5.22±1.67	99.91	0.16

Validation of Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

LINEARITY

The linearity of calibration curves in pure solution was checked over the concentration ranges of about 4-20 µg/ml for Atenolol and 4-20 µg/ml amlodipine. Appropriate aliquots from the standard stock solutions of Atenolol and amlodipine were used to prepare two different sets of dilutions Series A and B stock solutions as follows. Series A consisted of different concentration of Atenolol (4-20 µg/ml).Aliquot from the stock solution of Atenolol (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with 0.1 HCL to get

final concentration in range of 4-20 µg/ml. Series B consisted of varying concentrations of amlodipine (4-20 µg/ml). Appropriate volume of the stock solution of amlodipine (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with 0.1 HCL to get final concentration in range of 4-20 µg/ml. The calibration curve were constructed by plotting drug concentration versus the absorbance values.

PRECISION

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions for Atenolol and Amlodipine (5,10 ,15 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometric method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1Week for 3 different concentrations of standard solutions of Atenolol and Amlodipine (5,10, 15,µg/ml for both Atenolol and Amlodipine). The intra and inter-day accuracy and Precision were calculated and results were presented in the table-2. Precision of the analytical Method was found to be reliable based on %RSD (Table 2).

Table: 2 Precision studies by UV Method

Sr.no	Conc (µg/ml)	Sr.no.	Intraday precision(% RSD)		Intraday precision(% RSD)	
			Atenolol at 224 nm	Amlodipine at 232.8 nm	Atenolol at 224 nm	Amlodipine at 232.8 nm
1	5	1	0.385	0.330	0.385	0.330
2	10	2	0.384	0.327	0.383	1.333
3	15	3	0.384	0.326	0.384	1.336

Limit of detection (LOD), Limit of Quantification (LOQ)

Limit of detection was found to be 0.74 µg/ml for Atenolol at 224nm and 0.72 µg/ml for Amlodipine at 232.8nm respectively and Limit of Quantification was found to be 2.45µg/ml Atenolol at 224nm and 1.78 µg/ml for Amlodipine at 232.8 nm respectively.

ACCURACY

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120%. Results of assay and recovery were Presented in Table-3.

Table: 3 Recovery studies on the drugs

Sr.no	Name of the drug	Amount of sample (µg/ml)	Recovery Level	Amount of drug added (µg/ml)	Total amount found (µg/ml) ±SD	% Recovery	% RSD
1	Atenolol	10	80% 100% 120%	8 10 12	17.823 20.251 22.250	99.05 101.17 101.08	1.8 1.2 0.3
2	Amlodipine	1	80% 100% 120%	0.8 1.0 1.2	1.921 1.972 2.147	101.16 99.03 98.04	1.3 0.6 1.1

Table: 4 Regression data of Atenolol and Amlodipine

Sr.no	Parameters	Atenolol	Amlodipine
1	Wavelength(nm)	224	232.8
2	Beer's law limit (µg /ml)	2-22	2-34
3	Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.0135x +0.0169 0.0135 0.0169	y = 0.0174x + 0.0169 0.0174 0.0169
4	Correlation coefficient (r2)	0.9991	0.9976
5	LOD (µg/ml)	0.74	0.72
6	LOQ (µg /ml)	2.45	1.78
7	Precision (%RSD, n = 3) Interday Intraday	 0.384 0.384	 1.333 0.326
8	Accuracy (%Recovery, n= 5)	99.05- 101.09	99.02 - 101.16

RESULTS AND DISCUSSION

The two methods selected for multi-component analysis were given the satisfactory results. A novel approach to use 0.1N HCL 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 isoisorptive 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 0.9991 - 0.9976 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 99.05-101.16% justifies the accuracy of method.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of Atenolol and Amlodipine in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of Atenolol and Amlodipine and hence the Method was also economic for estimation of Atenolol and Amlodipine from tablet dosage Form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of Atenolol and Amlodipine in method, hence it can be Conveniently adopted for routine quality control analysis of the drugs in combined Pharmaceutical formulation.

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