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Estimation Of Atenolol In Bulk Drug And Tablet Dosage Form By Uv Spectrophotometric Method

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ABSTRACT

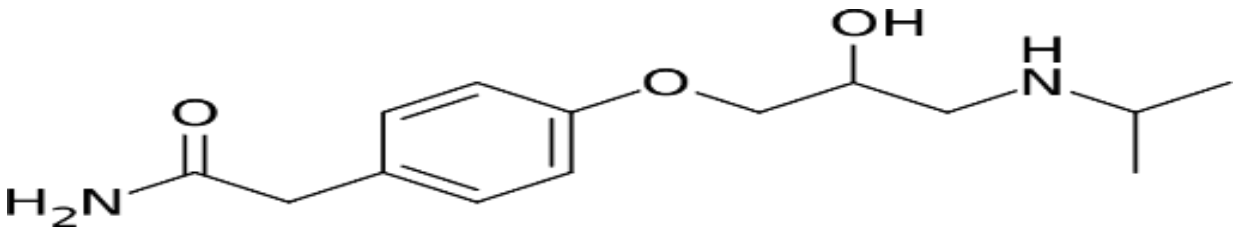
The aim of the present work was to develop and validate a simple, accurate and cost-effective UV spectrophotometric method for the routine estimation of Atenolol in bulk and tablet dosage form. The method was developed using distilled water as diluent and the absorbance was measured at 224 nm. Linearity was established at a concentration range of 2-12 µg/ml with a correlation coefficient (R^2) of 0.999. LOD and LOQ were found to be
1.214 µg/ml and 5.232µg/ml respectively. The method was validated according to ICH guidelines with respect to range, linearity, precision and accuracy. The results of all the parameters were found within the acceptable limits. It was found that additives present in marketed tablet dosage form did not interfere with the estimation of drug. The findings suggested that the method is found to be economical, quick and sensitive. Hence, the method was used for the routine quantitative analysis of Atenolol in tablet dosage form.

Key words: Atenolol, Antihypertensive, Spectrophotometry, Method validation.

Introduction

Atenolol is a β- adrenergic blocker, a drug of choice in the treatment and management of hypertension and also has anti-anginal and antiarrhythmic properties.[1] It is available in the market as tablet dosage form with brand names viz., Aten-25, Tenormin-25., Atiol-25, Tenolol-25 etc. chemically it is 2,2(1-methylethy) iminobis (2 hydroxy propane-3, 1-diyloxy- 4,1-phenelene) di acetamide.[2] A comprehensive literature survey revealed that there are few UV spectroscopic methods available for the estimation of Atenolol in combination and also alone but no method specified with distilled water as solvent. Hence, an attempt was made to develop a simple, accurate and cost-effective UV spectrophotometric method for the estimation of Atenolol in bulk and tablet dosage form. The validation parameters studied as per ICH guidelines were accuracy, precision, reproducibility, repeatability and robustness.

Figure 1. Structure of atenolol



Materials and methods

Materials

Atenolol was obtained as a gift sample from Zydus Cadila Mumbai. All the reagents used in this method were of analytical grade.

Methods

Preparation of standard stock solution [3]

The stock solution-I was prepared by dissolving 100 mg Atenolol in 100 ml distilled water and the concentration corresponds to 1mg/ml. 1mL of stock solution-I was diluted to 10 ml with distilled water to get concentration of 100 µg/ml.

Concentration range

The concentration range was established using 0.2 ml**,** 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml of stock solution-II and individually the volumes were made up to 10ml using distilled water in volumetric flasks to attain series of concentrations ranging from 2 to 12 µg/ml and sonicated for 5 min.

Determination of λ_{max}

A dilution corresponding to 6 µg/ml was used to determine the λ_{max} using UV spectrophotometer by scanning between the wavelength range of 200-400nm and the atenolol showed maximum absorption at 224 nm.

Preparation of sample solution [4]

Twenty tablets were weighed accurately and powdered. The Tablet powder equivalent to 50 mg of Atenolol was transferred into a 500 mL volumetric flask and dissolved in a little quantity of distilled water. Then the solution was sonicated for 30 minutes and filtered using Whatman filter paper No#41. The filtrate so obtained was diluted with distilled water to produce 500 ml. The resultant solutions were measured at a wave length of 224 nm against a reagent blank. The concentration of drug was calculated with the help of standard calibration curve.

ANALYTICAL METHOD VALIDATION

The developed method was validated as per ICH guidelines in terms of linearity, accuracy, precision, ruggedness and robustness

Linearity and Range:

The linearity was evaluated by linear regression analysis and the linearity was established within a concentration of 2-12 µg/ml. the regression equation and correlation coefficient are shown in fig.3 and table 2**.**

Accuracy:

The Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug solution in tablet samples within the analytical concentration range of the proposed method at three different levels of 80%, 100%, and 120%. After proper mixing, absorbencies were measured at 224 nm and the results were calculated in terms of %RSD. Results are shown in table 3.

Precision:

Intra-day Precision

Variation of results within the same day was analyzed. Intra-day precision was determined by analyzing the standard solutions of Atenolol (8, 10 and 12 µg/ml) at three different time intervals of the same day i.e. morning, afternoon and evening. The concentration range selected was on the basis of linearity range.

Inter-day Precision

Variation of results between the days was analyzed. Inter-day precision was determined by analyzing the Atenolol dilutions of (8, 10 and 12 µg/ml) in linearity range at three consecutive days. Both inter and intra-day precision determinations were carried out triplicate and average of three determinations was taken as mean and standard deviation was calculated.

Reproducibility [5]

Standard solutions of Atenolol 2-12 µg/ml were prepared and analyzed spectrophotometrically by Analyst 1 and Analyst 2, separately.

Repeatability [6]

Standard solutions of Atenolol 2-12 µg/ml were prepared and analyzed by spectroscopic method. The solutions were analyzed six times and the mean standard deviation and %RSD was calculated.

Ruggedness [7]

Ruggedness of the method was determined by studying the degree of reproducibility of results through changing the laboratory, instrument, glass apparatus and reagents (table 1). For doing so, we have approached Dr.H.L.T College of Pharmacy, Kengal, Channapatna. It was found that the instrument, chemicals and reagents were of different brands and these differences were utilized to study the ruggedness of the proposed method. There were no significant variations in the analytical data when compared to the established method. Two different brands of tablets were analyzed and the results were calculated in terms of % recovery and expressed as %RSD. Results are shown in table 5 & 6.

Table 1: Ruggedness parameters

Parameters	Laboratory	Name of the instrument	Manufacturer of the chemicals used
Lab. 1 with analyst I	M.M.U.College of Pharmacy, Ramanagara	SHIMADZU-(model:1700S, Japan) double beam UV-vis Spectrophotometer	Datta scientific work chemicals, Bengaluru.
Lab. 2 with analyst II	Dr. H.L.T.College of Pharmacy,kengal, Channapatna	Systronic UV-vis Double beam spectrophotometer	Loba chemicals, Mumbai

Robustness: [7]

Robustness of the method was validated by changing the P^H and temperature of reaction mixture before their estimation. The temperature of the reaction mixture was either raised by $+5^0$C (by warming) or reduced by -5^0C (by cooling) to study the effect of change in temperature on absorbance. To study the effect of change in P^H, the reaction mixture was treated with 2 drops of 0.1N NaOH and the other with 0.1N HCl solution. The experiments were repeated thrice and the data was used to calculate mean and standard deviation

Limit of detection and quantitation:[8-12] **Limits of detection (LOD):**

The LOD of the pure drug was estimated by analyzing the sample using a series of very dilute solutions under same experimental conditions. First the dilute solutions of several times less than the lowest concentration of Beer range were prepared and scanned over the optimum wave length of 224 nm. The lowest concentration of the analyte which showed absorption at given λmax was recorded and the experiment was repeated thrice for confirmation. The LOD for pure drug was found to be 1.224 µg/ml.

LOD was also calculated using a equation LOD = 3.3/S

Limits of quantitation (LOQ): [13-18]

The first approach is to determine LOQ by visual evaluation method. LOQ is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision.

The second approach determines the signal - to - noise ratio by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. LOQ isthe minimum concentration at which the analyte can be reliably quantified at the signal - to - noise ratio of 10:1.

The third approach is by using the equation LOQ=10/S

The slope S was estimated from the calibration curve of the analyte. The value of was estimated by calculating the standard deviation of the response obtained from the lowest concentration of the analyte.

Irrespective of the approach applied, the LOQ was subsequently validated by the analysis of a suitable number of samples prepared at the LOQ and by determining the precision and accuracy at the same level. Experimental determination of LOQ was found to be 5.222 µg/ml

RESULTS

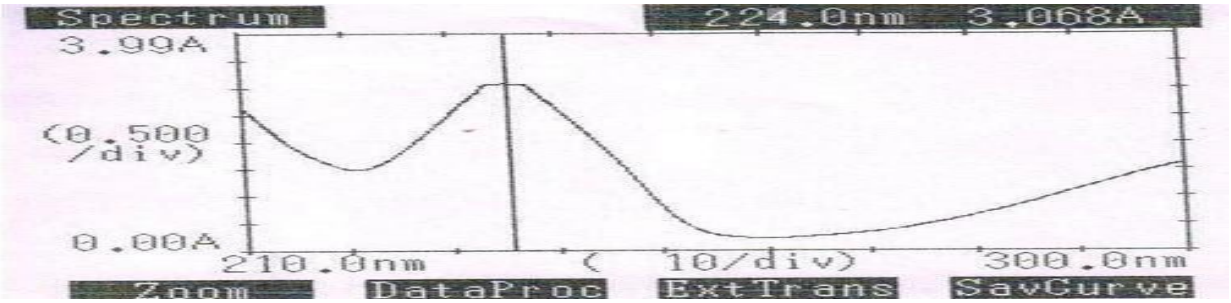


Fig 2. Absorption maxima of Atenolol at 224 nm

Table 2: Calibration curve data of Atenolol

Sl.No.	Concentration in µg/ ml	Absorbance ±SD* at λ_{max} 224 nm
1.	0.0	0.000
2.	02	0.108±0.125
3.	04	0.205±0.214
4.	06	0.310±0.157
5.	08	0.400±0.254
6.	10	0.510±0.214
7.	12	0.605±0.236

SD*=standard deviation (n=3)

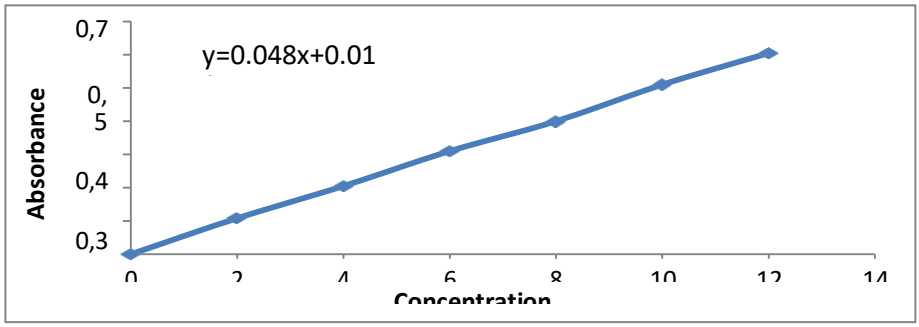


Figure 3. Standard calibration curve for Atenolol

Sl.	Conc. in(µg/ ml)	Inter-day absorbance Mean±SD*	% C.V	Intra-day absorbance Mean±SD*	% C.V
1.	08	0.412±0.001	0.38	0.410±0.016	0.63
2.	10	0.512±0.021	0.39	0.510±0.015	0.25
3.	12	0.616±0.011	0.66	0.610±0.022	0.26

. Table 3: Accuracy results for Atenolol

Brands	Initial amount (µg/ ml)	Amount of pure drug added (µ g/ ml)	Amount recovered (µg/ ml)	% Recovery ±S.D*
Aten-25	08 10 12	08(80%) 10(100%) 12(120%)	8.024 9.894 12.023	100.24±0.123 98.86±0.326 100.23±0.324

SD* =**Standard deviation** (n=6)

Table 4: Precision results for Atenolol

SD*=**Standard deviation**, n=3

Table 5: Ruggedness results for Atenolol

Sl.No	Brand	Label claim(mg)	Lab. 1* with analyst I		Lab. 2* with analyst II	
			Amount found (mg)	% Recovery±SD**	Amount found (mg)	%Recovery±S.D**
1.	Aten- 25	25	25.02	100.2±0.126	24.98	99.9±0.246

(Lab 1* MMU College of pharmacy, Lab 2* Dr. HLT College of pharmacy, **Average of six determinations, n=6)

Table 6: Robustness results for Atenolol

Type	Sl.No.	Conc. In (µg/ ml)	Change in temperature		Change in P^H	
			$+5^0C$	-5^0C	ops of 0.1N NaOH	2drops of 0.1N HCl
			Absorbance at 224 nm Mean±S.D*			
Pure Drug	1 2 3	08 10 12	0.402±0.024 0.521±0.028 0.608±0.011	0.406±0.014 0.510±0.012 0.609±0.028	0.401±0.006 0.512±0.006 0.605±0.055	0.406±0.026 0.521±0.021 0.608±0.040

S.D*=**Standard deviation**, n=3

Table 7: calibration data for Atenolol at 224 nm:

Parameters	Calibration data at 224 nm:
λmax	224 nm
Beer's law limit (µg/ ml)	2 -12µg/ml
Molar Absorptivity	$1.1162 Lmol^{-1}cm^{-1}$
Regression Equation(Y=a+bc)	Y= 0.052X+0.011
Slope (b)	0.01419 to 0.01465
Intercept(a)	- 0.004513 to 0.0016750
Correlation Coefficient (R^2)	0.999
Limit of detection (LOD)	1.214µg/ ml
Limit of quantitation (LOQ)	5.232µg/ ml

Table 8: Results of assay of tablet dosage form

Volume of stock solution used	Amount of drug (label claim) (µg/ ml)	Absorbance at 224 nm	Amount of drug found (µg/ml)	Percentage purity found ±S.D* (%w/w)
0.2ml	02	0.119	02.124	100.73±0.15
0.6ml	06	0.306	06.221	100.54±1.31
1.0ml	10	0.539	09.990	99.79±0.71
1.4ml	14	0.645	13.235	99.96±0.24
1.8ml	18	0.898	17.888	100.90±1.34

S.D*=**Standard deviation (n=3)**

DISCUSSION

An attempt was made to develop a simple UV spectrophotometric method for the estimation of Atenolol in pure and tablet dosage form. The process method development was initiated with the selection of solvent, water was chosen as solvent as the atenolol is soluble in water. A series of concentrations were prepared to establish the concentration range for the estimation. The concentration range established was found to be 2-12 µg/ml and the λmax was found to be 224nm under given experimental conditions (Fig.2&3 and table 2).

The proposed method was validated in accordance to ICH guidelines. The regression analysis results indicated R^2 value of 0.999, which explains the linear relationship between the concentration and absorbance.

Recovery studies were carried out by standard addition method. The percentage recovery of Atenolol from the tablets was found in the range of 99.94 – 100.30%. Here, the excipients of the tablet dosage form did not show any interference during estimation evidenced by high recovery efficiency and the method is said to be accurate (table 3).

Precision of the method among intra-day precision, inter-day precision, there was no significant variation in absorption readings and the percent coefficient of variations (% C.V) (table 4).

Ruggedness of the developed method was determined by changing the analytical tools such as laboratory, instruments, analyst and chemicals. No significant variations in the data was found (table 5).

Robustness of the method was established by slightly changing the temperature and P^H of the reaction mixture. The data so obtained showed no significant variation in the absorption pattern (table 6).

Limit of detection limit and limit of quantitation were determined from the standard deviation of response and slope of the calibration curve. LOD and LOQ of Atenolol were found to be 1.224µg/ml and 5.222µg/ml respectively (table 7), which describes the sensitivity of the method.

Atenolol (Aten-25) tablets were used for the assay. The amount of drug found was in the range of 99.80 – 100.80% (table 8).

Reproducibility of results was established by preparing and analyzing the standard solution of Atenolol by Analyst-I and analyst-II separately. Repeatability was determined by analyzing the sample at the given concentration and wave length for at least six times and it was found that the variability in the results was not more than 0.5%.

Based on above findings, it is clear that the proposed spectrophotometric method is found to be simple, cost effective, accurate, precise, rugged and robust.

CONCLUSION

A new spectrophotometric method was developed to estimate Atenolol in bulk and tablet dosage forms using distilled water as solvent. The values of recovery studies are satisfactory and are in close agreement with the label claims of the marketed product. Absence of significant variation in results of inter-day, intra-day precision, ruggedness and robustness makes the developed method accurate, precise, rugged and robust. Hence, the method is suitable enough to be used for the estimation of Atenolol in bulk and any other pharmaceutical dosage forms.

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