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UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF ASSAY OF ATENOLOL TABLET FORMULATION

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ABSTRACT

A simple, sensitive, specific and validated UV method has been developed for the quantitative determination of Atenolol in pure and tablet dosage form. The λmax was found to be 226 nm for assay. The linearity was found in concentration range of 0-150µg/ml. The correlation coefficient was found 0.999. The regression equation was found as y = 0.004x+0.007. The method was validated for linearity, accuracy, precision and System suitability. The LOD and LOQ for estimation of Atenolol were found as 2.088 &6.329 respectively. Recovery of Atenolol was found to be 99.12%.

KEYWORDS: Atenolol, UV Spectrophotometry, Validation, Beer's law.

INTRODUCTION

UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation.[1]

Beer -Lambert law

When beam of light is passed through a transparent cell containing a solution of anabsorbing substance, reduction of the intensity of light may occur. Mathematically, Beer- Lambert law is expressed as,

A=a b c

Where, A=absorbance or optical density

a=absorptivity or extinction coefficient

b=path length of radiation through sample (cm)

c=concentration of solute in solution.

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λmax), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal.

The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal proceduresof standard absorptivity value, calibration graph and single or double point standardization. In standard absorptive value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

Ctest= (Atest×Cstd)/Astd

Where Ctestand Cstdare the concentrations in the sample and standard solutions respectively and Atestand Astdare the absorbances of the sample and standard solutions respectively. For assay of substance/s in multi component samples by spectrophotometer.[2]

Description of Drug

Atenolol is a selective β1 receptor antagonist which used primarily in cardiovascular diseases.[3] Atenolol (M.W. 266.34), designated chemically as (C14H22N2O3), (RS)-2-{4-[2-Hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide (Fig.1). It is a white powder and

melting point 152°C to 155°C. It is sparingly soluble in water, soluble in ethanol, Slightly Soluble in methylene Chloride, practically insoluble in Ether. Atenolol drug is Optical rotation: +0.10° to -0.10° and dissociation constant (pKa) is 9.6 at 24^{o}C.[4] Beta-blockers are competitive inhibitors and interfere with the action of stimulating hormones on beta-adrenergic receptors in the nervous system and decrease the formation of Angiotensin II and secretion of aldosterone. Its effect on the bronchiolar smooth muscles is vasodilatation there by it helps in the treatment of cardiovascular disease such as angina, hypertension, cardiac arrhythmias and myocardial infractions.[5]

Aim of Present Work

This work deals with the validation of the developed method for the assay of Atenolol from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability.

The aim and scope of the proposed work are as under:

• To develop suitable spectrophotometric method for assay of Atenolol tablet.

• Perform the validation for the method

Experiamental

1. Material and Instrument

Analytical pure Atenolol drug was procured from Micro labs Ltd. Banglore (India). Ethanol (purity>99.9%), Methanol (HPLC grade), 0.1N Hydrochloric acid (HCL) and all other chemicals used were of analytical grade and obtained from Merck. Triply distilled water with conductivity value <0.3 □S cm-1 at 298.15 K was used for preparation of samples. Shimadzu double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements.

2. Selection of solvent and wavelength

The solubility of Atenolol was determined in different solvents viz. distilled water, methanol, ethanol, 0.1 HCL and phosphate buffer and it was found that the Atenolol showed the absorbance in range of 226-238nm. Absorbance spectrums of Atenolol in different solvents were studied carefully during Spectrophotometric technique. But among all these obtained spectra of Atenolol from different solvents, the spectrum with ethanol solvent was found to be better as Atenolol was stable in solvent, showed very clear absorbance at 226nm.

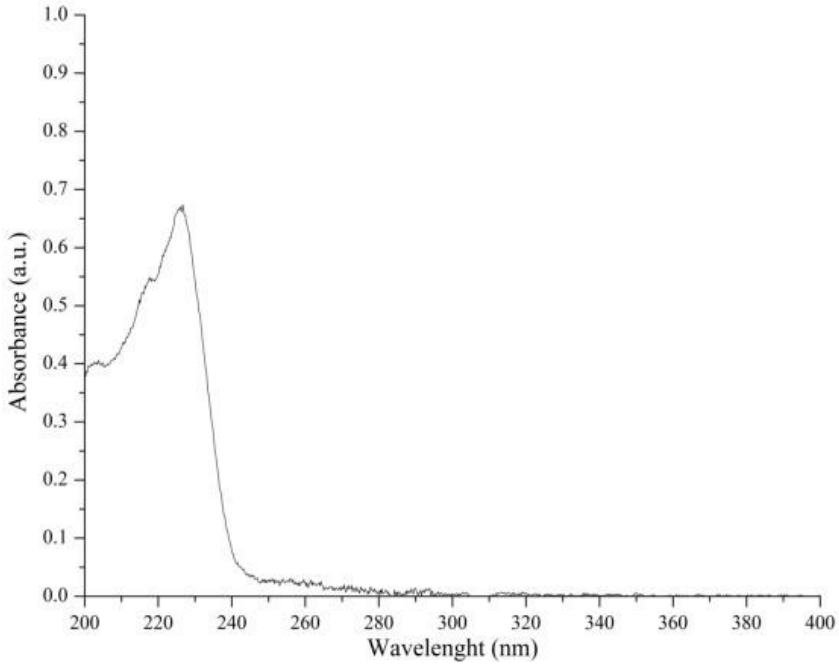

Fig 2: UV-spectrum of Atenolol in 0.1N HCL at λ=226nm.

3. Standard preparation

10 mg drug was dissolved in 0.01N HCL and was shaken well. Then 85 ml water was added to it to adjust the volume up to 100 ml (100 ppm). From that 5 ml was taken and volume was adjusted up to 50 ml with diluents.

4. Test preparation

20 tablets were weighed and powdered. Powdered tablet equivalent to 100 mg of Atenolol was weighed and taken into 100 ml volumetric flask then 15 ml of 0.1N HCL was added and shaken well to dissolve it after that 85 ml of water was added to adjust the volume up to 100 ml. From that 1 ml of solution was withdrawn and taken in 100 ml volumetric flask. The volume was adjusted with diluent up to 100 ml.

5. Assay Method

The assay is performed by single point UV-Visible Spectrophotometric Method The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

Ctest= (Atest×Cstd)/Astd

Where Ctestand Cstdare the concentrations in the sample and standard solutions respectively and Atestand Astdare the absorbances of the sample and standard solutions respectively.

VALIDATION

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

1. Accuracy
2. Precision
3. Detection Limit
4. Quantitation Limit
5. Linearity
6. System Suitability

1. **Linearity:** Six points calibration curve were obtained in a concentration range from 0-150 ppm for Atenolol. The response of the drug was found to be linear ininvestigation concentration range and the linear regression equation was y = 0.004x+0.007 with correlation coefficient 0.998.

2. Precision

Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were <0.98% and <0.79%, respectively.

Table 1: Precision study.

Sample No.	% Assay	
SET	Intraday	Interday
1	100.8	99.3
2	102.1	98.2
3	103.4	99.0
4	102.0	99.0
5	101.5	98.6
6	97.1	100.5
Mean	100.1	99.1
SD	0.98	0.79
%RSD	0.97	0.81

3. Accuracy

Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 150% is to be added in sample. The result shown that

best recoveries (98.27-99.12%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table 2: Accuracy Study.

% Recovery Level	% Recovery	Mean %Recovery	SD	% RSD
50%	97.62	98.27	0	0
	99.63		0.0057735	0.005854
	97.56		0.00816497	0.008279
100%	97.56	98.55	0.00693889	0.007041
	98.57		0.00942809	0.009566
	99.54		0.1503083	0.015251
150%	98.13	99.12	0.00707107	0.007134
	99.11		0.00707107	0.007134
	99.12		0	0

4. System Suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

Table 3: System Suitability Study.

Sample No.	Absorbance
1	0.66
2	0.661
3	0.66
4	0.66
5	0.66
6	0.66
Average	0.66
SD	0.000408
%RSD	0.061856

5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated directly from the calibration curve using the formula 3.3 σ/S and 10 σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of the intercept.

Table 4: Analytical characteristics of drugs by proposed Spectrophotometric technique.

Parameters	Spectrophotometric technique
sample Atenolol	
λmax, nm	226
Beers law limit, µg/mL	0-150
Linearity range, µg/mL	0-150
Regression equation (Y=mx + c)	y = 0.004x+0.007
Slope (m)	0.004
Intercept (c)	0.007
Correlation coefficient (R2)	0.998
Limit of quantification, LOQ (µg/mL)	2.088
Limit of detection, LOD (µg/mL)	6.329

CONCLUSION

The present analytical method was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The present analytical method can be used for its intended purpose.

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REFERANCE

1. ChatwalGR, Anand S (2002) Instrumental Methods of Chemical Analysis. (5thedn), Himalaya Publishing House, New Delhi.
2. Davidson AG (2002) Ultraviolet-visible absorption spectrophotometry. In Beckett AH, Stenlake JB, (4th edn), Practical Pharmaceutical chemistry. CBS Publishers and distributors, New Delhi, 275-278.
3. A.R.Tengli and B. M. Gurupadayya, (2013) "Chromatography Method Development and Validation of Tablet Dosage form Containing Losartan Atenolol and Hydrochlorthiazide Using Internal Standard, 4(5): 1–5.
4. J. Kavitha, S. Muralidharan, (2011) "Development and Validation of New Method for Atenolol, Hydrochlorothiazide and Losartan potassium by RP-HPLC: Its Application to Routine Quality Control Analysis," 2011; 3(2): 4–7.
5. N. R. Pai and S. S. Patil, (2013) "Development and validation of liquid chromatographic method for atenolol and its related substance," 4(2): 76–84.