Table 4: Standard Calibration curve of ethanolic solution of Atenolol

Concentration (µg/mL)	Absorbance (a.u.)
4	0.1±0.009
8	0.125±0.01
12	0.156±0.008
16	0.185±0.01
20	0.234±0.012
24	0.264±0.006
28	0.292±0.007
32	0.334±0.011
36	0.366±0.009
40	0.384±0.01
44	0.419±0.008
48	0.439±0.006

Data were expressed as means ±SD (n=3)

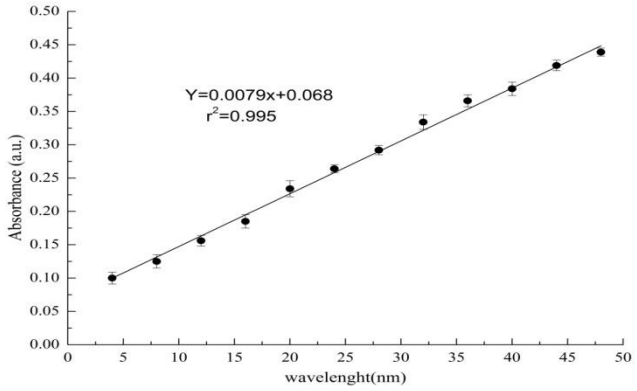

Figure 3: Calibration curve of ethanolic solution of Atenolol

2.11 Precision

Three times of three different concentrations (25, 50, 75 and 100µg/mL) were studied on the same day and the values of relative standard deviation (%RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision. Relative standard deviation (%RSD) is the absolute value of the coefficient of variation. The Relative standard deviation is calculated by equation: %RSD= Sx100/X, where S is a standard deviation, X is equal to mean. Interday and intraday precision of the Atenolol was performed. Data are shown in Table 6.

Table 6 Evaluation data for precision

Set	Inter day	Intra day
1	101.2	**99.1**
2	100.5	98.7
3	101.4	99
4	101.7	98.4
5	101.5	**98.9**
6	99.8	100.3
Mean	101.01	99.06
SD	0.725	0.653
%RSD	0.71	0.65

2.12 Accuracy

Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 25, 50, 75 and 100µg/mL is to be added in sample and Calculated the mean assay, Standard Deviation and % relative standard deviation (%RSD). The result that best recoveries of the spiked drug were obtained at each added concentration, indicating that the method was accurate (shown in Table 7).

Table 7: Evaluation data for accuracy

Amount added (µg/mL)	25	50	75	100
%Recovery	98.65	97.54	98.62	99.88
	99.56	99.58	99.56	99.06
	98.62	99.6	99.54	99.82
Mean recovery(%)	98.94	99.22	99.24	99.58
SD	0.534	0.575	0.537	0.457
%RSD	0.539	0.579	0.541	0.458

2.13 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 8)[6].

Table 8: Analytical characteristics of drugs by proposed Spectrophotometric technique

Parameters	Spectrophotometric technique
	Atenolol
λmax, nm	226
Beers law limit, µg/mL	4.0-48
Molar absorptivity,L/molcm	$7.7x10^6$
Linearity range, µg/mL	4.0-48
Regression equation (Y=mx + c)	Y= 0.0079x+0.068
Slope (m)	0.0079
Intercept (c)	0.068
Correlation coefficient (R^2)	0.995
Sandell's sensitivity (µg/cm^2/0.001 a.u.)	0.00003458
Limit of quantification, LOQ (µg/mL)	2.088
Limit of detection, LOD (µg/mL)	6.329
No. of data points	12
Degree of freedom	10
Pearson's r	0.997

3. HHLC analysis of Drug

3.1 Chromatographic Condition

The mobile phase containing Methanol: Water (70:30). The mobile phase was filtered on a 0.45 micron membrane filter and then Ultrasonicated for 30 min. The flow rate was set to 0.6 mL/min. Drug showed good absorbance at 226 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (28^0C). The composition and the flow rate of the mobile phase were programmed for the motor pump and delivered at a constant rate. The baseline was continuously monitored during this period. The peak area was recorded for every concentration by selecting the UV detector wavelength suitably so that there was less interference from mobile phase and to obtain highest sensitivity.

3.2 Stock Solutions and Calibration Curve

Standard stock solutions containing Atenolol was prepared by dissolving 100mg of atenolol in 60 mL of mobile phase. It was then ultrasonicated for 10 minutes and the final volume of solution was made up to 100 mL with mobile phase to get stock solutions containing 1 mg/mL. Calibration curve was prepared by taking appropriate 1mL and 0.5mL standard Atenolol in 10 mL volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 0.1 mg/mL and 0.05 mg/mL of Atenolol respectively. Standard solutions were injected through 10 µL loop system and chromatograms were obtained using 0.6mL/min. flow rate.

4. Results and Discussions

The research paper described the HPLC and doble beam UV-visible methods for determination of Atenolol.

4.1 Spectrophotometric Technique

The scaled method for the determination of Atenolol drug. The solutions were scanned in the wavelength range of 200-800 nm at appropriate dilution from stock standard solution. Atenolol had shown the best solubility (Table 2). The spectral characteristics were also good in this solvent and absorption maxima at 226 nm in ethanol. it was found that the system was suitable for drug and there was no major change. The Atenolol in ethanol as solvent predicted linear regression of absorbance on concentration (range 4 to 48 µg/mL) obtained the equation Y= 0.0079x + 0.068 with correlation coefficient (R^2) of 0.995.

Several analytical characteristics of drugs by proposed Spectrophotometric technique has been carried out (shown in table 8).

%RSD as repeatability were calculated which shown the best precision. Accuracy and precision has been shown in Table 1 and Table 2 respectively.

4.2. HPLC Method

The retention time of drug was determined by HPLC method. It was found about 5.03±0.029 min. Figure 4 predicted the chromatogram of Atenolol. For HPLC study a Prospers Start C18 (250 x 4.6 mm, 5 µm) column for separation at ambient temperature. This column provides efficient and reproducible separations of non-polar compounds while minimizing solvent usage. Initially various mobile phases were tested to obtain the best separation and resolution. The mobile phase consisting of methanol and water in the ratio of 70:30 v/v was found to have good resolution. The chromatographic conditions were optimized to achieve best separation and to get best resolution between analyses and to optimize chromatographic parameters like resolution, tailing factor and retention time (Fig.4).

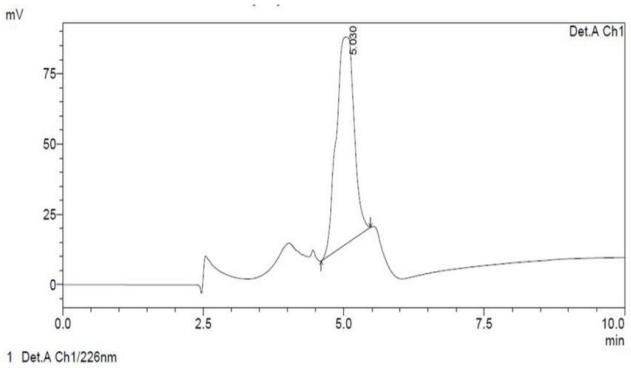

Figure 4: Chromatogram of Atenolol.

5. Conclusion

The validated Spectrophotometric and HPLC technique were found to be a simple, isocratic, precise, accurate, sensitive and reliable. Multicomponent analysis of Atenolol with ethanol, methanol has also supported and proved to be an efficient method as per ICH guidelines. The most striking feature of this method is its simplicity and rapidity, not requiring any sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. The described Spectrophotometric technique gives linear and precise results for determination of Atenolol. Validated isocratic HPLC methods have been developed for the determination of multicomponent dosage forms. Its chromatographic run time of 10 min allows the analysis of a large number of samples in a short period of time. Therefore, these methods can be scaled up for the routine analysis of pharmaceutical dosage forms and forensic application. Spectrophotometric technique was simpler however HPLC method was more sensitive and the same may be used as an alternative method when advanced instruments like HPLC are not available for routine quantification purpose.

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