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Method Development and Validation of Atenolol Drug by Spectrophotometric and HPLC Technique in Forensic Application

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Abstract: ***Atenolol is a selective β_1 receptor antagonist. The drug belonging to class of beta blockers used primarily in hypertension and cardiovascular diseases. It is highly misused drug and overdoses lead to death. The aim of this work was to develop a simple, isocratic, precise, accurate and sensitive Spectrophotometric and HPLC technique for determination of Atenolol. The HPLC technique is costly, caring and complicated so the applied method is novel, safe and sensitive for spectrophotometric estimation in UV-region. Then calibration curve was prepared in chosen solvents. The suitability, accuracy and precision studies were done by calculating SD and %RSD. The recovery of Atenolol was found near to 99.5 %. Validation of proposed methods was carried out for its suitable conditions according to guidelines and these methods are scaled up in pharmaceutical and forensic application.***

Keywords: Atenolol, forensics, HPLC, Recovery, Validation

1. Introduction

From past few years, the use of β_1 receptor antagonist drugs is restricted in sports by the both international federations and international Olympic committee [1] and highly abused drug. Atenolol is a selective β_1 receptor antagonist which is used primarily in cardiovascular diseases [2]. Atenolol (M.W. 266.34), designated chemically as ($C_{14}H_{22}N_2O_3$), (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 (pK_a) is 9.6 at 24°C [3].

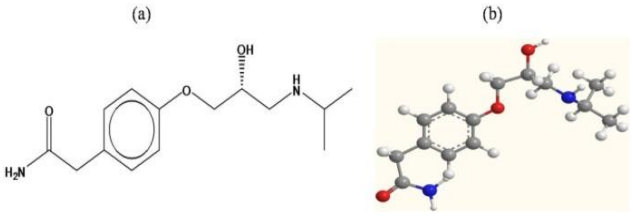

Figure 1: Atenolol (a) Molecular structure (b) Ball and stick model

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, an myocardial infractions [4]. But in other hand , it is a highly misused drug and overdose without prescription lead to death and In past few years, number of cases are coming up to forensic science laboratories related to drugs of abuse. Number of scientific publication have been observed on analysis of β-blockers groups using different methods such as gas chromatography method coupled with mass spectrometer, liquid chromatography with spectrophotometric detector[5]. The objective of this study was to develop and validate a simple, isocratic, accurate and sensitive UV-visible and HPLC methods using appropriate conditions for the determination of Atenolol drug.

2. Materials and Methods

2.1 Materials

Analytical pure Atenolol drug was procured from Paras pharmaceuticals Ltd. Baddi, Himachal Pradesh (India) and characterized by using FTIR technique. 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 μS cm^{-1} at 298.15 K was used for preparation of samples. Phosphate buffer 6.8pH used as guidelines of Indian Pharmacopoeia.

2.2 Instrumentation and analytical conditions

Shimadzu double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements. A Gradient HPLC PU 2080 Plus (JASCO) with UV- 2075 Plus detector and RPC18 column was used. A Rheodyne injector with a 10 μL loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing. Analytical balance was used for weighing the samples. All the chemicals and Whatmann filter paper (no.41) was used throughout the experimental work.

2.3 Solvent Selection

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 (Table 1).

Table 1: Evaluation data for solubility

Solvent	Wavelength (nm)
Water	238
Methanol	229
Ethanol	226
0.1N HCL	240
Phosphate buffer	230

2.4 Selection of Wavelength

An accurately weighed quantity 10 mg of Atenolol and transferred to 100mL volumetric flask a diluted with various solvents: Ethanol, Ether, 0.1N HCL, Methanol, Distilled water, Phosphate Buffer (PH 6.8) respectively. Then, determined the maximum absorbance of Atenolol in ethanol at 226 nm of Spectrophotometric technique (Fig.2).

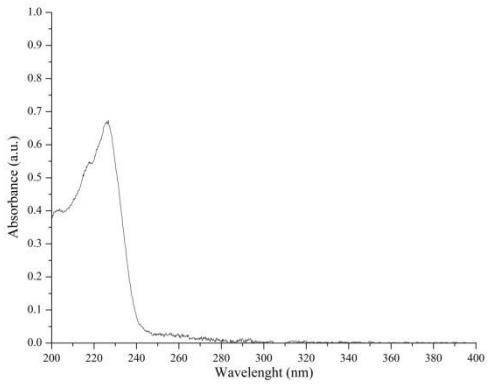

Figure 2: UV-spectrum of Atenolol in ethanol at λ=226nm.

2.5 Stability of Sample

The sample of 40µg/mL Atenolol solution was prepared by suitable dilution with diluents and absorbance were taken at 226nm against the blank. The stability of sample was found to be more than 10 hrs as predicted Table 2.

Table 2: Stability of sample

Atenolol (mg/mL)	Time(in hrs)	Abs. at 226nm
40	0	0.384
40	2	0.384
40	4	0.383
40	6	0.382
40	8	0.381
40	10	0.381
40	12	0.380

2.6 Suitability

System suitability was also determined and it was found that the system was suitable for drug and there was no major change. Suitability was determined between the six samples. Calculate the mean assay, Standard Deviation and % relative standard deviation (%RSD) (Table 3).

Table 3: Evaluation data for suitability

Sample	Absorbance (µg/mL)
1	0.81
2	0.811
3	0.81
4	0.81
5	0.812
6	0.811
Average	0.810
SD	0.008
%RSD	0.098

2.7 Preparation of standard stock solution for Validation of Spectrophotometric technique

A standard stock solution was prepared by dissolving 100mg of Atenolol in sufficient amount of Ethanol. It was then sonicated for 10 minutes and the final volume solution was made up to 100 mL with Ethanol to get stock solutions containing 1000 µg/mL.

2.8 Determination of absorptivity (1%, 1cm) and Molar absorptivity

Absorptivity (A) (1%, 1cm) and Molar absorptivity (ε) values of Atenolol were calculated using following formula, A (1%, 1cm) = Absorbance/Concentration (g/100mL), Molar absorptivity (ε) = Absorptivity × Molecular weight/10 respectively.

2.9 Sensitivity

Sensitivity of the proposed method for Atenolol was determined by calculating Sandell's sensitivity (SS) (µg/cm^2/0.001/abs. unit) using the relation SS = M/ε, where M and ε indicates the molecular weight and molar absorptivity respectively. Sandell's sensitivity means the smallest weight of the substance that can be detected in a column of solution of unit cross section.

2.10 Linearity and range

Calibration curves were prepared by taking appropriate aliquots of standard Atenolol stock solutions in 100 mL volumetric flask and diluted up to the mark with ethanol to obtain final concentration 4,8,12,16,20,24,28,32,36,40,44 and 48 µg/mL. Linearity of the drug was determined and found that the drug showed linear curve at various concentration. Calibration curve was constructed by plotting absorbance vs. concentration. Table 4 and Figure 3 predicted the calibration curve. Each experimentation was repeated three times.

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)

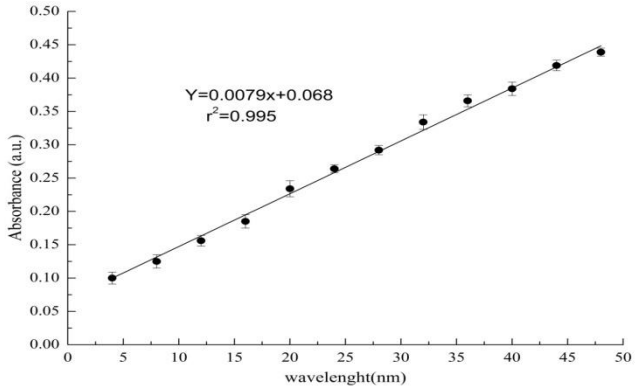

Figure 3: Calibration curve of ethanolic solution of Atenolol

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).

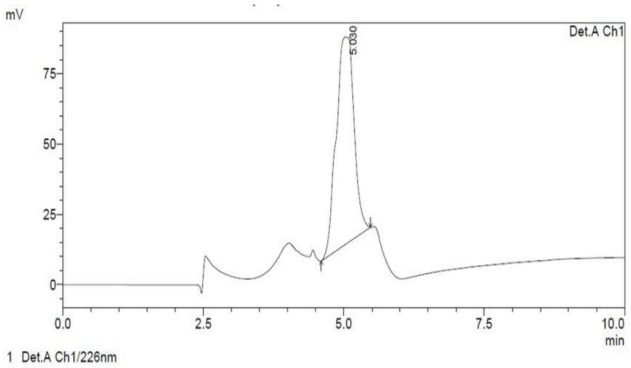

Figure 4: Chromatogram of Atenolol.

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.

References

[1] V.D.Toulet, V. Cirimele, J. P. Goulle., G. Pepin, and P. Kintz, “ß-adrenergic compounds through hair analysis,” *Probl. Forensic Sci.*, pp. 82–89, 2000.
[2] A.R.Tengli and B. M. Gurupadayya, “Chromatography Method Development and Validation of Tablet Dosage form Containing Losartan , Atenolol and Hydrochlorthiazide Using Internal Standard by RP-,” vol. 4, no. 5, pp. 1–5, 2013.
[3] J. Kavitha, S. Muralidharan, and B. Suresh, “Development and Validation of New Method for Atenolol, Hydrochlorothiazide and Losartan potassium by RP-HPLC: Its Application to Routine Quality Control Analysis,” vol. 3, no. 2, pp. 4–7, 2011.
[4] N. R. Pai and S. S. Patil, “Development and validation of liquid chromatographic method for atenolol and its related substance,” *Der Pharm. Sin.*, vol. 4, no. 2, pp. 76–84, 2013.
[5] E. Pufal, “Research on the determination of xenobiotics in epidermal products and their usefulness in forensic toxicology,” *Probl. Forensic Sci.*, pp. 38–49, 2004.
[6] P. P. Nath, K. Sarkar, P. Tarafder, and G. Paul, “Development of a visible spectrophotometric method

for the quantitative determination of metanil yellow in different food samples," *Int. J. Pharma Bio Sci.*, vol. 4, no. 2, pp. 685–692, 2013.

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