UNIVERSAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH (UJPSR)
Volume 10 Issue 1 Year 2026 Page No. 20 – 25
Estd. 1990

The analytical method development and validation of atenolol by using spectroscopic analytical grade solvent

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Article History
Received : 25th March 2026
Revised : 25th April 2026
Accepted : 25th June 2026
Published : 30th June 2026

Keywords
Atenolol, UV spectroscopy, Spectroscopic grade solvent, Method development, Validation

Abstract
Cardiovascular disorder is the most leading cause of death worldwide. India is also prone to cardiovascular disorders. To treat this disorder, many drugs have been developed out of which atenolol is one of the important drugs. Atenolol is a beta blocker having 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzene-acetamide nucleus in its structure. Atenolol binds to the beta receptor of the heart and decreases blood pressure, ultimately treating cardiac disorders. There are different methods for estimating atenolol conc. like HPLC, RP-HPLC, UP-HPLC, LC, GC, MS. But these techniques are more costly, complicated and require an export technician. To avoid these difficulties the UV method came, which is simple, accurate, specific, more precise and cost-effective. There are different UV methods for the estimation of atenolol, which require a solvent that may be costly. We know our body contains 85-90% water. This research explains the analytical method development and validation using a no-cost solvent, i.e., spectroscopic grade distilled water. Water is mostly present in our body & easily available outside, and solubility of atenolol in spectroscopic grade distilled water is good. So, we can use water as a solvent to determine the atenolol by UV, which shows good absorbance.

INTRODUCTION

Atenolol, chemically known as 4-[2-hydroxy-3-[(1-methyl-ethyl)amino]propoxy]benzene acetamide, is a selective β1-sympathetic receptor antagonist widely used in the treatment of cardiovascular disorders. It is commonly prescribed for the management of Hypertension, angina pectoris, myocardial infarction and arrhythmias. As a β-blocker, atenolol exerts its pharmacological effect by decreasing heart rate and reducing cardiac output, which ultimately leads to the reduction of blood pressure & myocardial oxygen consumption. Therefore, the precise and dependable quantification of atenolol in pharmaceutical formulation is crucial to ensure therapeutic effectiveness, patient safety & adherence to regulatory quality standards [1]. Hence, the development of a simple, precise, & cost effective for its routine analysis is of paramount importance in the pharmaceutical industry. Among the various analytical techniques available, UV-spectrophotometry remains one of the most widely used due to its simplicity, affordability, high sensitivity & rapid analytical turnaround. UV- spectrophotometric methods are particularly suitable for the routine analysis of drugs that exhibit characteristic UV-absorption. Atenolol shows a distinct absorption maximum(λmax) in the UV region, typically around 265nm, which makes it a suitable candidate for UV spectrophotometric determination [2].

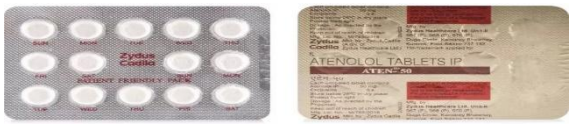

Figure 1: Atenolol Tablet IP

UV-spectrophotometry, compared to the analytical ways such as high-pressure liquid chromatography (HPLC), gas chromatography (GC), and mass spectroscopy (MS), does not require complex sample preparation or expensive equipment, which makes it an ideal choice for routine analysis, particularly in resource-limited settings. However, the reliability of the UV method depends heavily on its validation parameters, which ensure the method's capability to consistently produce accurate and precise results [3]. Method validation is a critical step in establishing the reliability & reproducibility of an analytical procedure. According to ICH guidelines, validation parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD) & limit of quantification (LOQ) must be properly examined to ensure method suitability for the needed purpose [4-7]. These validation

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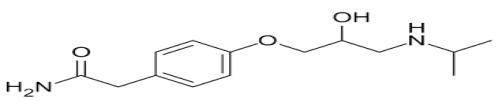
parameters are essential not only for quality assurance but also for meeting regulatory requirements, ensuring consistency across production batches & supporting the approval of pharmaceutical products by agencies such as FDA, EMA & WHO [8,9]. UV-Visible spectrophotometry operates on the principle that molecules containing a chromophore absorb UV/Visible light at specific wavelengths. The absorption is directly proportional to concentration. of substance as described by Beer-Lambert's law. Advantages of UV-spectrophotometry are cost-effective, simple, rapid throughput, non-destructive & sensitive [10]. The validated method will behave as a reliable tool for regular quality control analysis & regulatory compliance [11-15]. This validated method uses water as no cost solvent for analytical method development of atenolol [16-18]. This study centered on the growth & progress of a simple, precise, accurate, robust, & cost effective Ultra violet-visible spectrophotometric method for the quantitative estimation of atenolol in both its bulk drug and tablet dosage form. The necessity of the present study



is to develop and validate a UV-spectrophotometric method for the quantitative diagnosis of atenolol in bulk drug & tablet dosage form using no cost solvent [19-21].

Figure 3: Chemical structure of Atenolol

METHODOLOGY

Material & method

- **Instrumentation**

A double beam UV-Visible spectrophotometer (Shimadzu UV-1800) fitted with a matched quartz cell of 10mm path length was used for all spectroscopic measurements. Weighing of samples was performed using an electronic analytical balance (Shimadzu ATY224R). Spectroscopic grade distilled water was used as a solvent throughout the analysis. An ultrasonic bath sonicator (Model-3.5L capacity H) was employed to facilitate the preparation & dissolution of the sample solution.

- **Reagents and chemicals**

Atenolol's pure medication sample was generously provided by Zydus Healthcare Ltd. The ATEN 50 tablet formulation, which contains 50 mg of atenolol per tablet, was bought from the local market and produced by Zydus Healthcare Ltd., Unit II, Kumrek East, Sikkim 737132 Industries.

- **Preparation of standard stock solution**

To create the stock solution with a concentration of 1000 µg/mL, 100 mg of atenolol was dissolved in 100 milliliters of solvent with a pH of 7. The dissolution of the drug was enhanced by ultrasonication.

- **Preparation of sample stock solution**

In a 100ml conical flask, half-filled with distilled water, 100 mg of atenolol was dissolved and sonicated for 10 minutes. The solution was filtered into another volumetric flask. Then, the volume was made up with spectroscopic grade solvent and again sonicated for 10 minutes.

Determination of analytical wavelength

The UV spectrum's wavelength was chosen for the study of atenolol. Atenolol was synthesized at a concentration of 100 µg/mL, and its spectra was scanned between 200 to 400 nm to identify the maximum wavelength. The absorbance maximum against distilled water was discovered to be 265 nm.

- **Preparation of Calibration curve of Atenolol in spectroscopic grade distilled Water**

A series of 10 ml volumetric flasks was filled with aliquots of stock solutions ranging from 0.1 to 2.0 ml, and the volu me was adjusted with distilled water.

The range of 1, 2, 3, 4,...,20 µg/mL was serially diluted.

At λ_{max} 265 nm, the absorbance was measured.

UV Method Validation

- **Precision**

Three duplicates of each solution were taken, and the recovery % was computed. Studies of fluctuation within and between days showed how accurate the system was. Standard and sample solutions were measured three times in a single day for intraday research, and the %RSD was computed. In interday experiments, the % RSD was computed after three consecutive days of repeated measurements of the standard and sample solutions. Precision of the proposed analytical method for atenolol was assessed to determine its reliability. Method precision was evaluated in terms of repeatability (Intra-day precision) and reproducibility (Inter-day precision), both performed under the same laboratory conditions. The relative standard deviation (RSD) values obtained from these measurements are presented in the table below.

- **Accuracy (Recovery study)**

The three concentrations were selected analytical range and their absorption was measured & each concentration was analyzed in triplicate to assess the accuracy of the proposed method. Evaluation of accuracy through recovery studies using the standard addition technique was done. Known amounts of the standard drug were added at three different concentration levels- 80µg/mL, 100µg/mL, 120µg/mL. The obtained result indicated that the mean percentage recovery at all levels was within an acceptable range of 98-102%. For the recovery study, the prepared mixtures were transferred separately into 100ml volumetric flasks and diluted to volume with spectroscopic grade distilled water. The absorbance of each resulting solution was measured at the maximum wavelength(λ_{max}) against a

blank. Accuracy was further confirmed by analyzing atenolol at three different levels of 80%, 100%, 120%, with three replicates prepared for each level. After analysis, the percentage recoveries were calculated from the calibration curve & values obtained for atenolol were 97.68%, 98.52%, and 98.76% with relative standard deviation (RSD) values 0.460654, 0.572574, and 0.041338. Since all RSD values were below 2%, the result demonstrated good repeatability and confirmed the reliability of the analytical method.

- **Limit of detection (LOD) & limit of quantification (LOQ)**

LOD is the smallest conc. of the analyte that gives the measurable responses. LOD calculated as follows

LOD = 3.3* σ/s

Where σ = standard deviation of intercept

S = slope of the standard calibration curve

Limit of quantification (LOQ) is the smallest concentration. of analyte, which gives a response that can be accurately quantified. Calculated as following

LOQ = 10* σ/s

Where σ = standard deviation of intercept

S = slope of the standard calibration curve

- **Linearity**

The conc. range of 0-20 µg/mL shows linearity after proper validation. The calibration curve was plotted between concentration. Vs absorbance as per linear regression analysis. The r^2 value was determined.

- **Range**

Typically derived from a linearity study, the range was identified by determining the lowest to the highest concentration. of analyte.

RESULTS

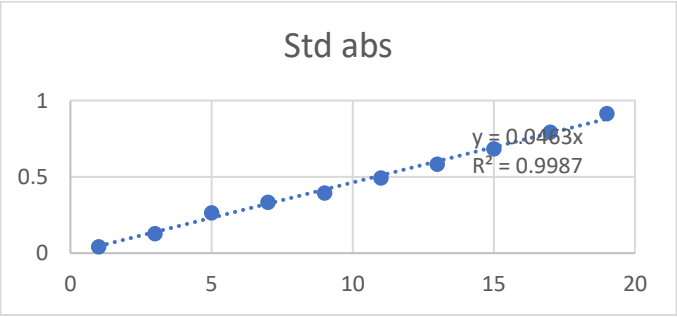
- **Linearity and range**

Table: 1

Conc	Std Abs	Test Abs
2	0.041	0.092
4	0.126	0.185
6	0.262	0.278
8	0.332	0.361
10	0.393	0.464
12	0.492	0.555
14	0.582	0.652
16	0.683	0.731
18	0.791	0.834
20	0.913	0.936

Table: 2

Conc	Std abs	Test Abs
1	0.041	0.046
3	0.126	0.139
5	0.262	0.232
7	0.332	0.324
9	0.393	0.417
11	0.492	0.51
13	0.582	0.6
15	0.683	0.694
17	0.791	0.787
19	0.913	0.871



- **Accuracy (Recovery Study)**

Table: 3

Original level (µg/ml)	Amount added (µg/ml)	Total amount	Amount recovered* (µg/ml)	%Recovery ± SD
5	4	9	8.907	97.68 ± 0.460654
5	5	10	9.926	98.52 ±0.572574
5	6	11	10.926	98.76 ±0.41338

- **Precision**

Table: 4

Statistical Parameters		2µg/ml		8µg/ml		18µg/ml	
Intraday	1	0.092	99.35205	0.367	99.08207	0.83	99.59203
	2	0.094	101.5119	0.36	97.19222	0.85	101.9918
	3	0.093	100.432	0.371	100.162	0.84	100.7919
	Mean	0.093	100.432	0.366	98.8121	0.84	100.7919
	S.D.	0.001	1.079914	0.005568	1.503176	0.01	1.199904
	R.S.D (%)	1.075269	1.075269	1.521247	1.521247	1.190476	1.190476
Interday	1	0.092	99.35205	0.367	99.08207	0.83	99.59203
	2	0.0948	102.3758	0.36	97.19222	0.85	101.9918
	3	0.094	101.5119	0.371	100.162	0.84	100.7919
	Mean	0.0936	101.0799	0.366	98.8121	0.84	100.7919
	S.D.	0.001442	1.557474	0.005568	1.503176	0.01	1.199904
	R.S.D. (%)	1.540834	1.540834	1.521247	1.521247	1.190476	1.190476

Table: 5

Statistical Parameters		1µg/ml		9µg/ml		19µg/ml	
Intraday	1	0.046	0.993521	0.417	100.0719942	0.871	99.01102649
	2	0.045	0.971922	0.416	99.83201344	0.87	98.89735137
	3	0.047	0.993521	0.416	99.83201344	0.871	99.01102649
	Mean	0.045667	0.986321	0.416333	99.91200704	0.870667	98.97313478
	S.D.	0.000577	0.01247	0.000577	0.13855298	0.000577	0.065630359
	R.S.D. (%)	1.264271	1.264271	0.138675	0.138675005	0.066311	0.066311287
Interday	1	0.047	101.511879	0.411	98.63210943	0.88	100.0341025
	2	0.046	99.35205184	0.42	100.7919366	0.86	97.7606002
	3	0.047	101.511879	0.414	99.35205184	0.87	98.89735137
	Mean	0.046667	100.7919366	0.415	99.59203264	0.87	98.89735137
	S.D.	0.000577	1.246976823	0.004583	1.099730188	0.01	1.136751165
	R.S.D. (%)	1.237179	1.237179148	1.104235	1.104235107	1.149425	1.149425287

- **Limit Of Detection (LOD)**

By using a calibration curve, the suggested method's limit of detection for atenolol was established.
The LOD was found to be 0.549573

- **Limit Of Quantitation (LOQ)**

By using a calibration curve, the suggested method's limit of quantitation for atenolol was established.
The LOQ was found to be 1.665373.

- **Discussion**

The calibration curve for atenolol followed by Beer-Lambert's law with a linearity range of 0-20 µg/mL conc. range., showing linear relationship between absorbance & conc. The r^2 value was found to be 0.9966, showing excellent linearity in graph-1. The method demonstrated good specificity with no interference, like excipients or solvents, at the given wavelength. With recovery values of (97.68, 98.52, 98.76) for successive concentrations of 9 µg/mL, 10 µg/mL, and 11 µg/mL and SD values less than 1%, as shown in Table no. 6. Precision studies, both intra & inter day, showed low %RSD value, confirming the repeatability & reliability of the method given in table-5&6. Accuracy was evaluated through a recovery study, where %recovery ranged between 97-99%, showing an accurate method free from matrix interference, as shown in Table 3. The method also exhibited satisfactory robustness and ruggedness, as minor changes in experimental conditions (such as wavelength variation or solvent change) did not significantly affect the absorbance values. Overall method suitability for routine estimation of atenolol in bulk & tablet formulations. The simplicity, speed & reliability make it particularly useful in pharmaceutical quality control laboratories.

- **Conclusion**

The UV-spectroscopy method was developed & validated as per ICH Q2(R1) guidelines.
The method involves measuring the absorbance of atenolol at its maximum wavelength (λ_{max}), typically around 265nm. using no-cost spectroscopic-grade distilled water as solvent. The method showed excellent linearity in 0-20 µg/mL concentration with an r^2 value of 0.9987, indicating an excellent linear relationship between concentration and absorbance. LOD & LOQ were found to be 0.549573 & 1.665373, respectively, demonstrating the method sensitivity for determining low concentrations of atenolol. Accuracy was determined at different conc level, i.e. 80%, 100%, 120% with a recovery range of 98-102%, confirming the method's reliability in quantifying atenolol in pharmaceutical preparations. Precision expressed with less than 2% RSD value, showing excellent reproducibility, robust and rugged. The method also demonstrated good specificity, with no interference observed from excipients present in formulations. This was confirmed by the consistent absorbance readings. In conclusion, the validated UV-spectroscopic method is precise, accurate, linear, specific, Sensitive, rugged & robust. This method is well-suited for daily analysis of atenolol in bulk & finished dosage form with simplicity, speed & cost effectiveness.

Funding: This research received no external funding.
Interest: The author declared no conflict of interest

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