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DEVELOPMENT AND VALIDATION UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ATENOLOL IN PURE MATERIALS AND PHARMACEUTICAL DOSAGE

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ABSTRACT

The current research aimed to develop and validate a simple, accurate and sensitive analytical method using spectrophotometer device in assaying raw material of atenolol and determination the content of atenolol in pharmaceutical tablets. Firstly, a solution of atenolol in hydrochloric acid (0.1N) was prepared and its absorption spectrum was examined in UV range against blank solution containing only hydrochloric acid (0.1N). The method was validated for linearity through preparation a series of certain concentration and measure their absorptions and correlation coefficient ,molar absorptivity, and Sandell's sensitivity were calculated. Method was detected for accuracy by performing recovery studies. The precision of the method was expressed as RSD of series of measurement by replicate estimation of drug. The proposed method was both evaluated for specificity by noticing any effects of common excipients that may add to atenolol pharmaceutical formulation and applied to commercial tablets The results indicated that the solution of atenolol in hydrochloric acid (0.1N) exhibiting λ max at 225 nm. The relationship between the concentrations and the absorbance was linearity with good correlation coefficient (0.9979) The molar absorptivity is $7.163\times10^{3}Lmol^{-1}cm^{-1}$and Sandell's sensitivity is $0.034\mu g/cm^{2}$. The method is appropriate over concentration range of 5 – 40µg/ml. The results of studying the validation of this method showed that the method have been validated statistically. The proposed method had its accuracy, precision, specificity, when applied in measurement atenolol in its pharmaceutical dosage forms. As a result we can use the proposed analytical method in determination atenolol in raw material and its commercial tablets.

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INTRODUCTION

Atenolol is considered as β-1 heart adrenergic receptor hindering drug. It doesn't steady on the cell membrane and doesn't have any activity as fractional agonist in other parts of the body. It is used in the treatment of Hypertension and Ischemic Heart Disease. Only about 50% of atenolol is absorbed from intestinal tract and most of absorbed drug arrives to the systemic circulation. It takes about two to four hours to arrive to its Peak blood levels after absorption. The occurring breakdown of Atenolol in liver is very sparing. This drug is defecated through urinary tract. Above than 85% of intravenous dose is excreted through the urine after 24 hours, but only about 50% after oral dose. A small amount of drug (about 6-16%of the absorbed amount) is assured to plasma proteins. It has an elimination half-life about 6 to 7 hours and there is no change in kinetic profile of atenolol after continuing administration. The drug arrives to peak plasma levels within 5 minutes after intravenous administration. The declination from peak levels is quick (5to 10 doubling) during the first 7 hours. The pharmacological effects as β block and anti-hypertensive continues for at least 24 hours after oral doses of 50 mg or 100 mg. Atenolol accrues in patients whom have renal symptoms and the dosage should be commuted for patients whose creatinine clearance is less than 35 mL/min/1.73m^2[1-3].

Atenolol has the chemical structure of benzene acetamide,4-[2 – hydroxy – 3 –[(1- methyl ethyl) amino] propoxy] as shown in Figure (1). The molecular weight of atenolol is 266. It is classified as poor polar hydrophilic compound and its solubility in water about 26.5 mg/mL at 37°C. It has a log partition coefficient (octanol/water) (log p= 0.23). It is freely soluble in 1N HCl (300 mg/mL at 25°C) and less soluble in chloroform (3 mg/mL at 25°C) [4].

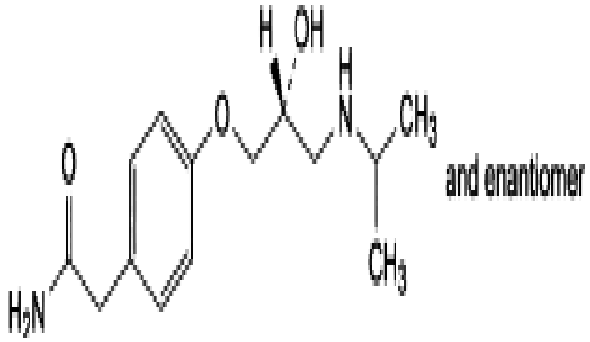


Figure (1): chemical structure of atenolol.

There are numerous analytical methods used in the measurement of atenolol in both pure material and pharmaceutical dosage forms. A high performance liquid chromatography (HPLC with UV detection) was reported by the United States Pharmacopeia (2003) for assay atenolol in its commercial tablets [5- 8]. The method suggested by British Pharmacopoeia (2001) involves UV spectrophotometry using methanol as a solvent [9].

The main goal of this study is to develop and validate a simple, accurate and sensitive analytical method using UV spectrophotometer for assay of atenolol in raw material and its pharmaceutical tablets using hydrochloric acid. Throughout this method we can both reduce money expenditures and save taken time in atenolol analysis in raw materials and dosage forms.

MATERIALS AND METHODS:

Pure atenolol, three commercially available tablets were purchased from the local market in Syria (A, B, C), hydrochloric acid, distilled water. In addition to instruments like UV-Vis spectrophotometer (Shimadzu, model 1800).

Preparation of standard solution:

100 mg of pure Atenolol was weighed and transported into 100ml volumetric flask and dissolved in hydrochloric acid (0.1N). The flask was agitated and volume was completed to standard line with hydrochloric acid (0.1N) to get a solution containing 10 µg/ml of Atenolol. The absorbance of the consequential solution was tested in UV range of UV spectrophotometer device to get the wavelength of maximum absorption (λ max), after using a blank of hydrochloric acid (0.1N).

Validation of the proposed method:

Linearity and Method sensitivity (LOD and LOQ):

The linearity of the studied method was verified by gauging the absorbance of six concentrations covering the range (5, 10, 20, 30, 35 and 40µg/ml). The absorption of each concentration was measured three times. Then the curve of absorbance against concentration was observed optically and statistically by calculating correlation coefficient.

To determine the values of LOD and LOQ using SD (standard deviation of response) and S (the slope of the calibration curve), the two equations:were used:

LOD = 3.3 * SD/S - LOQ = 10 * SD/S.

In addition, Molar absorptivity and Sandell's sensitivity were calculated.

Accuracy:

The absorption of three concentrations in the studied range were measured and each of them was replicated three times to evaluate accuracy of the studied method. To do that, recovery studies using standard addition method were performed. Standard drug aliquots at three different levels viz. 5µg/ml, 20 µg/ml, 30µg/ml, were added to reference amount of drug. These mixtures were moved into separate 100 ml volumetric flasks and then hydrochloric acid (0.1N) were added to each flask. Then, the absorbance of the consequential solutions was examined at λ max against blank prepared in the same way without addition of the examined drug.

Precision:

Intraday and Interday precision were determined by measuring the absorption of three different concentrations (5, 20 and 30 µg/ml) of atenolol. The absorption of each concentration was measured three times. Precision of the proposed method is expressed as SD or RSD of series of measurement by replicate estimation of drugs by this analytical method.

Specificity:

The specificity of the proposed method was evaluated by noticing any effects of common excipients that may add to atenolol pharmaceutical formulation such as starch, magnesium stearate, and lactose. To perform this purpose, a combination was made by addition equal amounts of starch, magnesium stearate, lactose, and atenolol. A quantity of the prepared combination was dissolved in hydrochloride acid(0.1N) with good agitating, filtered and the volume was completed to 100 ml with hydrochloride acid(0.1N). Precisely measured aliquots of atenolol filtrate were moved into separate 100 ml volumetric flasks and the contents of each flask were shaken thoroughly and each mixture was completed to 100 ml with hydrochloride acid(0.1N). The absorbance of the resulting solutions were measured at λ max against blank prepared in the same manner without addition of the examined drug.

Application to pharmaceutical dosage form (tablets):

A known weight equal to 100 mg of the drug triturate was taken after the broken down of ten tablets of each brand. Then this amount was thawed in 50 ml hydrochloric acid(0.1N) with good agitating for about 10 minutes and filtrated to detach excipients. Then The filtrate solution was transported into 100 ml volumetric flask and diluted with hydrochloric acid (0.1N) in order to get (5, 20, 30) µg/ml. The absorbance of the consequential solution was measured at λ max against blank prepared in the same way without addition of the examined drug . The absorption of each concentration was measured three times and the percentage of recovery was determined from the calibration curve.

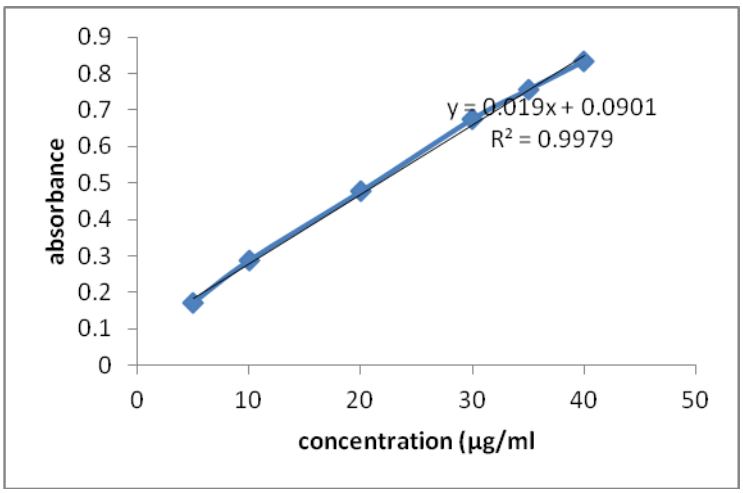
RESULTS AND DISCUSSION:

The absorption spectrum of the drug was tested in UV range UV spectrophotometer device against a reagent blank. The maximum absorption of the drug in the studied range is at λ max 225 nm.

Validation of the proposed method

Linearity and Method sensitivity (LOD and LOQ):

The calibration curve was built by charting concentration of atenolol versus absorbance (Figure 2). A linear relationship was found between absorbance and the prepared concentration over the series of 4-50µg/ml.



Figure(2): Calibration curve of atenolol in hydrochloric acid (0.1N).

The correlation coefficient, intercept and slope for the calibration data for atenolol were determined using the least squares method. It was found that this method proved its linearity within Beer's law limits in the range of (5 – 40 µg/ml), with good correlation coefficient (0.9979). and good molar absorptivity (7.63×10^{3} L $mol^{-1}cm^{-1}$)(table 1).
The limit of detection (LOD=0.67 µg/ml) and the limit of quantitation (LOQ=2.05) (table 1).

Sandell's sensitivity is the concentration of the drug in µg/ ml which will show an absorbance of 0.001 in 1 cm cell, and is expressed as µg cm^{-1} and it equals 0.034 µg/cm^{2} using this method (table 1).

Table (1): statistical data of the regression equation for atenolol in hydrochloric acid (0.1N).

Parameters	Atenolol Spectral Data
λ max nm	225
Beer's law limits, µg/ml	5 – 40
Molar absorptivity L $mol^{-1}cm^{-1}$	7.63×10^{3}
Sandell's sensitivity µg/cm^{2}	0.034
Limit of detection µg/ml (LOD)	0.67
Limit of quantitation µg/ml (LOQ)	2.05
Regression equation	Y=0.0901+0.019X
Intercept (a)	0.0901
Slope (b)	0.019
Correlation coefficient (r)	0.9979

Y= a + bX, where Y is the absorbance, a intercept, b slope and X concentration in µg/ml

Accuracy:

Accuracy of the proposed method was verified by doing recovery studies using standard addition method. A pure standard drug at three different concentrations within Beer's range was added to a known amount of atenolol which taken from dosage form. The total concentration was determined by the studied method. The determination with each concentration was replicated three times and average percent recovery of the added standard was calculated and results are tabulated in (Table 2). The results obtained showed excellent mean recovery percent values (99.9, 99.95, 99.5) % for (5 µg/ml, 20 µg/ml, 30 µg/ml) concentrations consecutively, so these values very close to 100%. In addition, values of standard deviation are very small (0.07, 0.05, 0.04). Consequently, these results indicate high accuracy of the proposed analytical methods.

Table (2): results of recovery study for atenolol.

Origin level (µg/ml)	Amount added (µg/ml)	Amount recovered* (µg/ml)	% Recovery ± SD
10	5	4.995	99.9±0.07
10	20	19.990	99.95±0.05
10	30	29.987	99.95±0.04

* Mean value of three determinations.

Precision:

To evaluate the precision of the used analytical method, means of three times measurements of the tested drug within Beer's law limits were taken. Relative Standard deviation of a series of measurements usually used as expressed of the precision of analytical method. Intraday and interday precision were evaluated using three concentration and three replicates of each concentration. The values of relative standard deviation were (0.22%, 0.23%, 0.049%) for (5 µg/ml, 20 µg/ml, 30µg/ml) concentration consecutively, when intraday precision was done. Also the values of RSD were very small (0.18%, 0.06%, 0.026%) for (5 µg/ml, 20 µg/ml, 30µg/ml) concentration consecutively, when interday precision was done. These results show good repeatability and reliability of our analytical method (table 3).

Table (3): evaluation of precision of the analytical procedure of atenolol.

Statistical Parameters		5 µg/ml	20 µg/ml	30µg/ml
Intraday	1	4.995	20.011	29.991
	2	4.992	19.985	30.015
	3	5.013	19.921	30.020
	Mean recovery	5	19.972	30.008
	Mean% recovery	100	99.86	100.02
	S.D.	0.011	0.046	0.015
	R.S.D. (%)	0.22	0.23	0.049
Interday	1	4.990	20.011	29.992
	2	5.009	19.988	29.980
	3	4.996	19.991	29.996
	Mean recovery	4.998	19.996	29.989
	Mean% recovery	99.96	99.98	99.96
	S.D.	0.009	0.012	0.008
	R.S.D. (%)	0.18	0.06	0.026

Specificity:

The results show that there isn't any effects of commonly used additives and excipients in the preparation of tablets on the determination of atenolol, as the values of recovery were (99.8, 99.94, 99.94) for (5 µg/ml, 20 µg/ml, 30 µg/ml) concentrations consecutively and the values of SD less than 1% (Table 4). So, the proposed method indicate its specificity.

Table (4): results of specificity study for atenolol.

Concentration(µg/ml)	Amount recovered*(µg/ml)	% Recovery ±SD
5	4.990	99.8±0.11
20	19.989	99.94±0.15
30	29.982	99.94±0.18

* Mean value of three determinations.

Application to pharmaceutical dosage form (tablets):

The assay of atenolol commercial tablets(brand A, brand B, brand C) was determined using the current UV spectrophotometric conditions and the results are shown in (table 5). The recovery values of atenolol determination in tablets were between (99.92 to 99.96 for brand A), (99.30 to 99.83 for brand B), (98.64 to 99.52 for brand C) and RSD values are not more than 1%. The assay values for the formulations were very close as mentioned in the label claim, indicating that there isn't any effects of commonly used additives and excipients in the preparation of tablets on the determination of atenolol.

Table(5): results of application of UV spectrophotometric used method in atenolol determination in pharmaceutical tablets.

Brand	Label claim(µg/ml)	Amount recovered*(µg/ml)	% Recovery± SD
A	5	4.996	99.92±0.011
	20	19.986	99.93±0.09
	30	29.990	99.96±0.1
B	5	4.965	99.30±0.12
	20	19.967	99.83±0.09
	30	29.930	99.76±0.06
C	5	4.932	98.64±0.16
	20	19.890	99.45±0.11
	30	29.856	99.52±0.09

* Mean value of three determinations

CONCLUSION

An accurate UV spectrophotometric analytical method was developed and validated for estimation of atenolol through dissolving in hydrochloric acid (0.1N). The method was suitable to determine concentrations in the range of 5-40µg/ml precisely and accurately. The limits of detection and quantitation for the drug was (0.67, 2.05). Furthermore, the values of RSD can be considered to be very satisfactory. The values of recovery studies for samples were very close to the amount of drug that is written on each brand. Finally, we can say that we can use the proposed method instead of other analytical methods, when the analysis and the quality control of atenolol in pure form and in pharmaceutical dosage forms is required.

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CONFLICTS OF INTERESTS:

There is no conflicts of interest involved in this article.

LIST OF ABBREVIATIONS

N	-Normality
Λ	-wavelength
$Lmol^{-1}cm^{-1}$	-liter/mol. Centimeter
$\mu g/cm^2$	-microgram/ square centimeter
UV	-Ultraviolet
SD	-Standard Deviation
RSD	-Relative Standard Deviation
LOD	-Limit Of Detection
LOQ	-Limit Of Quantitation

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