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METHOD DEVELOPMENT AND VALIDATION OF ATENOLOL USING TWO HPLC SYSTEMS

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Atenolol; Hypertension; RP- HPLC; Optimization; Method validation

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ABSTRACT: A reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of Atenolol (ATN) in bulk and pharmaceutical formulation. The method was optimized selecting chromatographic conditions of 60: 40 acetonitrile: water, Inertsil_ column(ODS-3 250 mm × 4.6 mm 5 μm), 20 μL injection volume, flow rate of 1 mL/min at ambient temperature (30 °C), and 276 nm. Using two HPLC systems of first HPLC system coupled with PDA detector and the second HPLC system coupled with UV detector showed no big difference in the method results. The method was validated giving good precision (RSD% < 1), acceptable linearity (R^2 > 0.998), and low LOD and LOQ (0.5 and 1.5 μg/mL, respectively) on both systems. Successful application on pharmaceutical dosage tablet form gave high recovery of 97.7%. The proposed method is economic, simple, and rapid and hence can be employed for routine analysis in quality control laboratories.

INTRODUCTION: Atenolol (ATN) is chemically described as 2-{4-[2-Hydroxy-3-(isopropylamino) propoxy] phenyl} acetamide (**Fig. 1**). It is white powder and water soluble. Atenolol is a selective β1 receptor antagonist, a drug belonging to the group of beta blockers class of drugs used primarily in cardiovascular diseases. Atenolol is used to treat angina (chest pain) and hypertension (high blood pressure). It is also used to treat or prevent heart attack or heart damage after a heart attack [1, 2]. Atenolol is available in oral dosage forms viz. tablet, syrups and capsules. In the tablet dosage this drug is commonly available in three different strengths *i.e.* 25, 50 and 100 mg. Atenolol is also available as an antihypertensive treatment in the form of compound preparations with diuretics [3].

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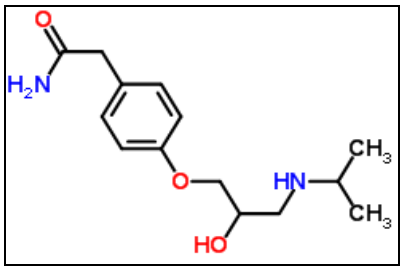


FIG. 1: CHEMICAL STRUCTURE OF ATENOLOL

In reviewing the literature, various analytical methods were found for the determination of atenolol whether alone or in its combination with other drugs in pharmaceutical preparations including spectrophotometry [4-6] kinetics [7, 8] Titrimetric [9] GC [10] and HPLC [11-15].

The aim of the present study was to develop of isocratic HPLC method for the analysis of ATN employing two HPLC systems of first HPLC system coupled with PDA detector and the second HPLC system coupled with UV detector. A further objective was to compare the sensitivity of a photo diode array detector (PDA) and UV/Vis detector for the determination of atenolol.

The analytical method was validated according to international conference on harmonization (ICH) guidance for validation of analytical procedure by examining the precision of the HPLC instruments used in this study, the linearity of the calibration curve and calculating the limit of detection (LOD) and the limit of quantification (LOQ) [16].

MATERIALS AND METHOD:

HPLC systems: Two HPLC systems were used during this study;

- First HPLC system (A) comprised a Flexar auto sampler -no Peltier, Flexar Binary pump, Inertsil® (ODS-3 250 mm x 4.6 mm 5 µm siloxan), and a Flexar /S275/S200EP Photo Diode Array detector (PDA) coupled with chromera® version 3.4.0.5712 software. Flexar column oven was connected with a system to control the column temperature.

- Second HPLC system (B) comprised a Flexar auto sampler -no Peltier, Flexar Quaternary pump, Inertsil® (ODS-3 250 mm x 4.6 mm 5 µm siloxan), and a Flexar UV/Vis detector coupled with chromera® version 3.4.0.5712 software.

Chemicals: Pharmaceutically pure sample of atenolol drug was obtained from Awamedica company (Erbil city - Kurdistan region of Iraq). Commercial tablet of ATN (100 mg) was brought from the local drug market. Acetonitrile and water were HPLC grade from merck KGaA, 64271 Darmstadt, Germany.

Preparation the mobile phase: The mobile phase of HPLC analysis was prepared from organic solvent of acetonitrile and water (v:v%). The mobile phase was degassed using an ultrasonic bath (100W, 80 KHz, Elmasonic P- Germany.).

Stock and working solutions: Stock solution of 1000 µg/mL of atenolol was prepared by dissolving 0.1 g of sample in 100 mL of acetonitrile. This stock standard solution was stored in a refrigerator and used to prepare the working solutions at different concentrations.

Sample solution preparation: Ten tablets were crushed to fine powder. An accurately weighed portion of the powder (equivalent to 20µg/mL) was taken and dissolved in 100mL solvent of acetonitrile.

Development and optimization of the HPLC method: To construct a basic background for developing an HPLC method with fast analysis for the determination of ATN, an isocratic programming was employed by analyzing solution of 10 µg/mL at 60%, (v/v) of ACN. The chromatographic conditions were set using a 20 µL injection volume, flow rate of 1 mL/min, column oven at ambient temperature (30 °C) and UV detection at a wavelength of 276 nm.

Method Validation:

Assessment the precision for HPLC systems: The precision of the two HPLC systems was evaluated by repeated injections (n=5) of concentration of 5 µg/mL ATN calculating the relative standard deviation (RSD %) of the peak area of ATN.

Linearity assessment: Set of ATN standards in the range of 2-10 µg/mL was prepared and injected into two HPLC systems and the detector response (peak area) plotted against the nominated concentration to generate the calibration curve. Using Excel® software, from the regression line, the coefficient of determination (R^2) was obtained to statistically assess the linear relationship.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of ATN in solution were calculated statistically based on the data from the calibration curve [17]. The calibration curve at low levels of ATN was used to statistically estimate the ATN peak area, which was significantly different from the blank peak area. The equation used to estimate this peak area was:

$$\text{LOD peak area} = yB + 3\,sB$$

Where yB is the blank peak area and sB is the standard deviation of the blank peak area. In practice, the value of yB and sB can be estimated from the calibration graph at the lower range by using values from the regression analysis of variance. Therefore, the value of yB was replaced

by the intercept value of the regression equation of the calibration graph of ATN at the lower range of 2-10 µg/mL. Similarly, the term sB was replaced by the residual standard deviation, which is the square root of the error mean square. Both terms were estimated using the regression function in Excel® software. The statistical data consist of the intercept, slope and standard deviation of the regression line (SD), after calculating the LOD and the LOQ peak area depending on the above information of the regression line, the LOD and the LOQ were determined according to the following equations:

LOD Peak area = intercept + 3 SD
LOD= (LOD Peak area – intercept) / slope
LOQ Peak area = intercept + 10 SD
LOQ= (LOQ Peak area – intercept) / slope

Application of the method to dosage form: The accuracy of proposed method was ascertained on the basis of average recovery % to determine ATN in its pharmaceutical preparation as tablets (equivalent to 20µg/mL) by triplicate injections under the same chromatographic conditions of the proposed method. The recovery was calculated according to the below:

% Recovery = (Recovered amount x 100)/Injected amount.

RESULTS AND DISCUSSION:

Development and optimization of method: Selecting best HPLC system through optimizing the chromatographic conditions is initial and important step prior to determination of atenolol in its commercial pharmaceutical dosages.

In this study, RP-HPLC method was developed and optimized using two HPLC systems connected with Inertsil®column(ODS-3 250 mm x 4.6 mm, 5 µm ciloxan). First HPLC system is coupled with PDA detector and the second HPLC system coupled with UV detector. Commonly, the UV detectors used for HPLC are single wavelength detectors for quantitative analysis. Whilst for more qualitative and quantitative information about the sample, photo diode-array detectors (PDA) may be employed to measure the absorbance at multi-wavelengths simultaneously.

The HPLC chromatograms in **Fig. 2** show the analysis of 10 µg/mL solution of ATN using two HPLC systems under isocratic chromatographic conditions.

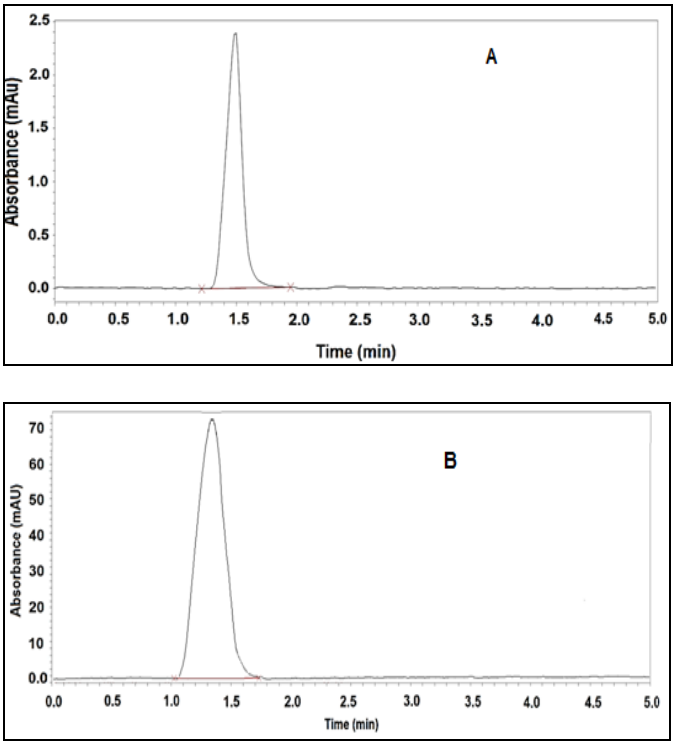


FIG. 2: CHROMATOGRAMS OF ATN PEAK USING TWO HPLC SYSTEMS: A (PDA DETECTOR) AND B (UV/VIS DETECTOR)

As shown in **Fig. 2** both chromatograms indicate a good determination of ATN peak at 60% strength of the mobile phase, although chromatogram B showed broadening, peak of ATN was appeared at approximately 1.5 min retention time under the same chromatographic conditions for both systems. A possible explanation for broadening might be to large injection volume, molecular weight of drug and build-up of contamination on column inlet, residual silanol interaction and column deterioration [18].

The best chromatographic parameters for the proposed method showed no difference between both HPLC systems as summarized in **Table 1**.

Method validation: Validation of the analytical method prior to determining ATN in dosage sample was done by examining such parameters of precision, linearity, and LOD and LOQ using two HPLC systems as follows:

Assessment of the precision for HPLC systems: Validation of the method was performed through examining the precision. The two HPLC systems achieved good precision reporting RSD% values of the peak area of ATN less than 1% as shown in **Table 2**.

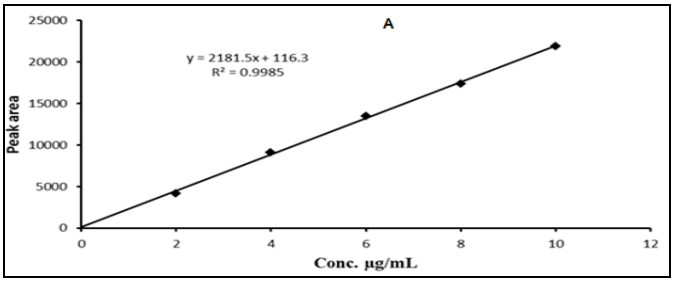
TABLE 1: CHROMATOGRAPHIC CONDITIONS OF PROPOSED ATN METHOD USING TWO HPLC SYSTEMS

Parameter	HPLC System A	HPLC System B
Column	Inertsil® (ODS-3 250 mm x 4.6 mm 5 µm siloxan)	Inertsil® (ODS-3 250 mm x 4.6 mm 5 µm siloxan)
Detector	PDA	UV
Wavelength detection	276 nm	276 nm
Mobile phase	60 % CAN	60% ACN
Flow rate	1 mL/min	1 mL/min
Chromatographic run	5 minutes	5 minutes
ATN retention time	~ 1.5 minutes	~ 1.5 minutes
Injection volume	20 µL	20 µL
Column temperature	30 °C	30 °C

TABLE 2: RSD% VALUES OF FIVE REPLICATE INJECTIONS OF 5 µg/mL OF ATN ON TWO SYSTEMS

Injection No.	Peak area of HPLC system A	Peak area of HPLC system B
1	9396	343201
2	9406	343795
3	9476	342956
4	9398	339675
5	9337	346665
Mean	9403	343258
STDEV	49.41	2491.4
%RSD	0.52	0.72

Linearity of the calibration curve:
As shown in **Fig. 3**, the results illustrate a good linearity between the peak area and the concentrations of the standard solutions of ATN on both HPLC systems giving coefficient of determination (R^2) > 0.998 over the selected range of concentrations.



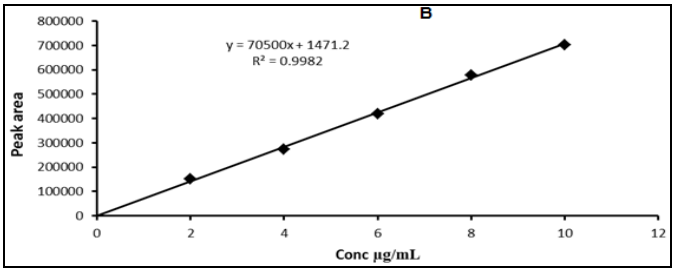


FIG 3: CALIBRATION CURVES OF ATN STANDARDS ON TWO HPLC SYSTEMS: A (PDA DETECTOR) AND B (UV/VIS DETECTOR)

LOD and LOQ determination: LOD and LOQ were calculated by calibration curve at low level of the concentrations. The results in **Table 3** show close values of LOD and LOQ of both systems.

TABLE 3: LOD AND LOQ VALUES OBTAINED FROM THE CALIBRATION CURVE OF LOWER CONCENTRATIONS (2-10 µg/mL) OF ATN OF BOTH SYSTEMS

Parameter	HPLC system A	HPLC system B
Slope	2181.45	70500.1
Intercept	116.3	1471.2
SD	313.62	10830.9
LOD peak area	1057.17	33964.02
LOQ peak area	3252.55	109780.62
LOD	0.4 µg/mL	0.5 µg/mL
LOQ	1.4 µg/mL	1.5 µg/mL

In the present method, two HPLC systems were operated HPLC coupled with photo diode array detector and HPLC coupled with UV/Vis detector. It can be concluded that the two HPLC systems offered acceptable precision, linearity and LOD and LOQ values despite the difference in the specification of the PDA and UV/Vis detectors. However, the PDA detector has the ability to monitor multi wavelength and present entire spectra of all peaks during chromatogram run and

monitor the peak purity as well [19]. The HPLC system coupled with PDA detector was selected for application the present method on pharmaceutical formulation sample.

Recovery: The validity and reliability of proposed method was assessed by recovery study. Determination of the ATN drug in its pharmaceutical dosage gave recovery equal to 97.7% with RSD% value of 0.49 indicating that the proposed method is suitable and reliable for the determination of ATN in pharmaceutical dosage forms.

TABLE 4: THE PERCENT RECOVERY (N= 3) OF 20 µg/mL ATN DOSAGE SAMPLE

Peak area of recovered amount (conc. 20 µg/mL)		Peak area of injected amount (conc. 20 µg/mL)	Recovery%	Mean of Recovery	RSD%
Injection 1	42137	42879	98.26	97.70	0.49
Injection 2	41792		98.46		
Injection 3	41756		97.38		

CONCLUSION: Using two HPLC systems (HPLC- PDA and HPLC-UV), an isocratic RP-HPLC method for the determination of atenolol was developed and validated. Optimizing the method using an Inertsil (ODS3 C18, 4.6x250 mm, 5µ) column offered reliable chromatography conditions of 20 µL injection volume, 1 mL/min flow rate, 30 °C column oven temperature and retention time of approximately 1.5 min at a run time of 5 minutes on both systems and 60% ACN of mobile phase strength with 276 nm wavelength detection.

Validation the developed method using both HPLC systems offered acceptable precision (RSD %< 1), good linearity ($R^2 > 0.998$) and low values of LOD (~ 0.5µg/mL) and LOQ (~ 1.5 µg/mL).

It can be concluded that the method shows no difference between both HPLC systems. HPLC system coupled with PDA detector was used to apply the present method on pharmaceutical formulation sample giving high recovery (97.7%).

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