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Development and Validation of New Analytical Methods for Estimation of Antihypertensive Drug

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ABSTRACT:-

Analytical technique development and authentication play an significant role in the discovery, development and manufacture of pharmaceuticals. A well-developed technique should be simple to authenticate. The broad variety of equipment, columns, eluent and operational parameters involved makes HPLC technique development a complex procedure. Method development requires collection of HPLC technique, the selection of proper technique for the analysis of a compound depends upon diverse factors like the nature of the compound to be analyzed, its solubility and other factors. Sample preparation, selection of chromatography: reverse, normal, or ion exchange. A further mode of HPLC i.e. gradient or isocratic is, Column dimension, selection of detector, flow rate, injection volume, pressure, the temperature of the column is finalized. For the proposed work Atenolol Drugs was selected for the present work. This drug was identified based on UV scan analysis. During Method development for the assay of Atenolol drug, various chromatographic conditions were followed; based on observation best-suited condition was considered and finalized.

After the development of the methods, they were further validated against various parameters like linearity, specificity, accuracy, intra-day as well as inter-day precision, robustness as per the guidelines suggested by the ICH. All statistical values (% RSD, % Recovery, Correlation Coefficient, Cumulative RSD) calculated were within the acceptance limits. The proposed methods for assaying anti-hypertensive drugs after validation were subjected to marketed preparations and the results obtained after analysis of the marketed preparations complies with the results of the standard drugs. Thus, these techniques can be further utilized during the routine study of the drugs in API's as well as in finished products form.

Keywords: Method Development, Validation, ICH guideline, Anti-hypertension Drug, Atenolol

I. INTRODUCTION

The main goal of pharmaceutical plant is to consistently produce products that meet the necessary standards and quality, while minimizing costs as much as possible. Analytical Chemistry refers to a scientific field that employs advanced technologies in analyzing and identifying the composition of substances using various techniques. Analytical chemistry involves researching and understanding the process of separating and quantifying different compounds or elements in both natural and man-made materials. It also involves identifying the various chemical components present in these materials. Analytical chemistry is splits into two primary divisions. Qualitative examination involves identifying the chemical components present in a sample, while quantitative examination determines the quantity of a specific element or compound in the substance or samples. **[1]**

The numbers of medications entering the market is growing all year. These medications could be also entirely new substances or modified versions of active ones. Medications are expected to be readily available in a form that ensures their quality and ability to be absorbed by the body, reaching the necessary concentration in the blood for a sufficient period of time. They should also have a timely onset of action, be administered in the correct dosage, and be safe, effective, and stable throughout their storage. The development of a drug is a lengthy process that involves several stages such as drug discovery, laboratory experimentation, initial testing on animals, human clinical trials, and gaining approval from regulatory authorities. In order to enhance the effectiveness and safety of the medication once it is approved, various authoritarian agencies such as the US

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Foods and Drugs Administration (USFDA) also mandate that the drug product undergo evaluation for its identification, strength, properties, quality, stability, and purity prior to its release for use. Therefore, it is crucial to have proper pharmaceutical validation and process controls to address any problems that may arise.

1.1 High Performance Liquid Chromatography

Chromatography a method of separation, was invented by the Russian botanist Tswett. He provides a thorough explanation of the procedure for separating pigments. This involves filtering them through a column and then further refining them using solvents. HPLC, which stands for High Performance Liquid Chromatography, is currently experiencing rapid growth as an analytical method for drug analysis. Its simplicity, strong specificity, and broad sensitivity range make it perfect for analyzing various medications in both types of doses and bodily fluids. Based on IUPAC, chromatography is a technique that physically separates or distributes components between fixed and moving phases.HPLC consigns to a type of liquor chromatography in which the liquor mobile phase is pushed from side to side the column at a high velocity. This leads to a significant reduction in analysis time compared to traditional column chromatography, by about 1-2 levels of magnitude. Additionally, it enables the use of smaller particles of the adsorbent or support material, greatly enhancing the efficiency of the column. The significance of chromatography is growing quickly in pharmaceutical analysis as it is being used more often to accurately distinguish, selectively identify, and quantitatively determine compounds that are very similar in structure. The purity of final products and intermediates can be determined using chromatographic methods, which is another important area where these methods are applied. The method is popular because it is sensitive and can be easily adjusted for accurate quantitative measurements. It is also suitable for separating non-volatile or delicate species, and it can be used on a broad range of substances that are important to the industry. Sensitive detectors have revolutionized liquid column chromatography by making it a fast, effective, precise, and extensively detailed method of separation. The significant expansion in chromatography can be attributed mostly to the preamble of a versatile method known as high-pressure liquid chromatography or frequently referred to as high-performance liquid chromatography.

1.2 Types of HPLC

1) Normal phase chromatography: In this type the immobile phase is polar, while the mobile phase is non-glacial. In this method, non-polar substances move more quickly and are released first due to the weaker attraction between these substances and the stationary phase. Polar composites take a longer period to elute due to their stronger attraction to the motionless phase. Consequently, this method is typically avoided in pharmaceutical functions as most drug molecules have polar characteristics, leading to prolonged elution periods.

2) Reverse phase chromatography: Reversed phase mode is widely used for separating important compounds in various fields such as biology, pharmaceuticals, chemicals, food, and biomedical engineering for both analytical and preparative purposes. The solid stage used in this process is a water-repellent material, which consists of a silica gel bonded with octyl and octadecyl functional groups. The liquid stage, on the other hand, is a solvent that is polar in nature, often consisting of water or a mixture of water and other solvents. Substances that have polarity tend to favour the moving phase and are the first to be removed during elution. As the solutes become more hydrophobic, there is an increase in retention. Typically, when the mobile phase has lower polarity, its eluent strength is increased

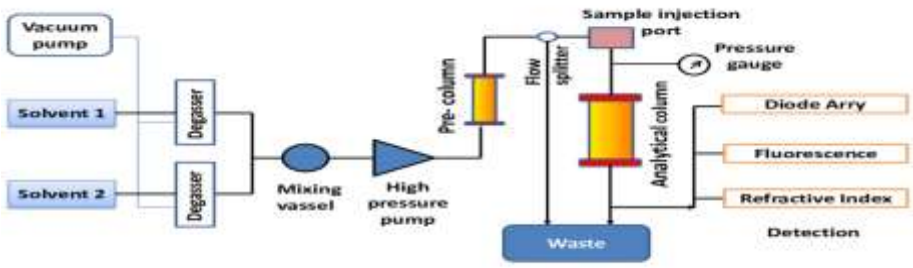

Fig. Reversed phase chromatography

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The pump offer a consistent and strong pressure without any fluctuations, and it can be set to change the makeup of the moving substance while carrying out the separation process. Detectors use a variation in refractiv index, UV-VIS amalgamation, or fluorescence following stimulation through an appropriate wavelength in order to identify the isolated substance.

1.3 Composition of a liquid chromatography system:

- Solvint
- solvent release system
- injectors
- samples
- columns
- diode array
- devastate collector
- data collections

1.4 Introduction to Method Validation:

Validation refers to the act of gathering proper documentation to ensure that a particular activity will constantly yield the wish outcome or products, meet up the determined requirements. Method validation involves proving that analytical practices are appropriate for their intended purpose as well as that they confirm the effectiveness, purity, authenticity, and strength of both the drug materials and the drug products. The primary objective of the validation procedure is to test the effectiveness of the technique and establish the acceptable range of variations for the conditions required to execute.

1.5 Performance parameters of method validation:

1) **Specificity:** Specificity refer to the capability to accurately determine the presence of the specific analyte even when potentially interfering substances are present. Typically, these could consist of contaminants, deteriorations, framework, and so on. all through the validation process of tests, the identification of impurity and measurement accuracy, it is crucial to carry out an examination on specificity. The method employed to illustrate specificity will vary based on the planned aim of the methodical process.
2) **Linearity:** The capability to get test outputs, which are directly relative to the absorption of analyse in the sample.
3) **Variety:** The analytical procedure's range refers to the span between the highest and lowest amounts of the substance being analyzed in a sample. This includes both of these amounts, and it is necessary to show that the procedure accurately, precisely, and linearly analyzes within this range. The specified range is typically determined through linearity studies and is based on the intended use of the method.
4) **Accuracy:** Correctness of an analytical procedure refers to how closely the value found in the analysis matches the accepted true value or reference value. Sometimes, it can be referred to as a measure of authenticity or accuracy. The specified range of the analytical process must be used to determine the level of accuracy
5) **Precision:** The precisions procedure measures how closely the measurements get from several samples of the identical homogeneous sample beneath specific conditions agree with each other (degree of variation).
6) **System appropriateness Testing:** The system needs to undergo testing to determine if it is suitable for its intended reason. System appropriateness testing is a necessary component of numerous analytical practices. The tests are designed with the belief that the instruments, electronics, analytical procedures, and samples to be studied form a complete system that can be assessed together.

II. ADOPTED METHODOLOGY

2.1 Equipments and chemical

2.1.1 Equipments and Apparatus used:

Table : Equipments and Apparatus

S.NO	NAME OF EQUIPMENT	MAKE AND MODEL
1	HPLC Instrument	G4288C 1220 Infinity II Gradient LC System VL
2	UV-Vis Spectrophotometer	HPLC UV Agilent
3	Sonicator	Ultrasonic bath sonicator ATS-1
4	Weighing machine	Digital mettle analytical balance Mettle Me204
5	Membrane filter	0.2 micro meter ,PTFE GL14

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Table : Solvents and Reagents

S.NO	Solvents	Source
1	Methanol	LOBA chemi cal pvt ltd
2	Water	LOBA chemical pvt ltd
3	Acetonitrile	LOBA chemical pvt ltd

2.2 Preliminary studies:

Preliminary studies conducted on the chosen drugs i.e. Atenolol comprised of melting point determination , UV-Visible spectroscopic studies.

Determination of Melting point:

The melting point of drug samples was examined by utilizing melting point apparatus. For this, little quantity of drug sample was occupied in the capillary tube close up from one sided as well as put in a melting point apparatus along with the temperature at which drug melts was note down.

2.2.1 UV-Visible Spectroscopy study:

2.2.1.1 Finding of λ_{max}:

The solution of drug sample i.e. Atenolol (50 µg/ml) was prepared, as well as scanned in the range of 226.99 nm to obtain the UV spectrum.

2.2.2 Method Development by HPLC

From the exhaustive literature examination, it was revealed that the selected drugs were successfully determined and validated in their API form by UV, HPLC and RP HPLC methods.

2.2.3 Method development for Atenolol:

Following conditions were developed for the estimation of Atenolol.

S.NO.	Mobile phase used	Composition	Wavelength	Flow rate	Resolution
1	Water: Acetonitrile (Isocratic)	50:50	276 nm	1ml/min	Average
2	Methanol: Water (Isocratic)	50:50	276 nm	1ml/min	Good (used for further study)

Table: Condition develop for estimation of Atenolol

S.NO.	Parameter	Conditions
1	Stationary phase	Agilent TC-C18(2),4.6x250mm,5um
2	Mobile. phase	Methanol: Water
3	Mobile phase ratio	50: 50
4	Detection .Wavelength	276 nm
5	Flow Rate,	1.0 ml/min
6	Sample Volume	20µl
7	Temperature	Ambient
8	LC System	Agilent test system and OpenLab CDS2
9	LOD	26.7848 ug/m
10	LOQ	81.1660 ug/ml

Table: - Condition develop for estimation of Atenolol

Every system appropriateness parameters were inside the range as well as satisfactory as per ICH rules. Thus, the adopted method was considered as an optimized method.

2.3 Validation of developed methods:

2.3.1 Prepare of Standard Stock solutions for estimation of Atenolol :

Standard stock solutions for the drug was prepared and then validated.

A. Diluent: Based up on the soluble of the drugs, diluent was chosed, methanol and water was obtain in the ratio of 50:50 for Atenolol.

B. Prepare of Standard stock solutions: Exactly weight 50mg of Atenolol transferred 50 ml of volumetric flasks, 50.0ml of diluents was put in and sonicated for 30 minutes.

C. Prepare of Standard working solutions : 50µl of Atenolol from stock solution was pipetted out and acquire into a 10ml volumetric flask and made up with diluent. (50µg/ml of Atenolol).

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D. Prepare of Standard Working solutions: 1ml of Atenolol taken from 50µg/ml of stock solution and add the 1ml of methanol made up the stock solution (25µg/ml).
E. Prepare of Standard Working solutions : : 1ml of Atenolol taken from 25µg/ml of stock solution and add the 1ml of methanol made up the stock solution (12.5µg/ml).
F. Prepare of Standard Working solutions: 1ml of Atenolol taken from 12.5µg/ml of stock solution and add the 1ml of methanol made up the stock solution (6.25µg/ml).
G. Prepare of Standard Working solutions: 1ml of Atenolol taken from 6.25µg/ml of stock solution and add the 1ml of methanol made up the stock solution (3.125µg/ml).
H. Prepare of Standard Working solutions: 1ml of Atenolol taken from 3.125µg/ml of stock solution and add the 1ml of methanol made up the stock solution (1.5625µg/ml).

2.4 Validation of Adopted Method:

2.4.1 Specificity:

Specificity refers to the checking of the interfering in the improved method. No interfering peaks were seen in blank and placebo at retention times of selected drugs in this technique. So, this technique was said to be specific. Specificity for all stock solutions obtained from the drugs were determined.

2.4.2 Linearity:

To calculate the linearity as well as range of the technique, dissimilar standard solutions were organized by diluting the standard stock solution with acetonitrile of concentrations: 20, 10, 5, 2.5, 1.25 µg/ml. Three shots from every concentration were investigated under the similar conditions. Linear regression examination was utilized to estimate the linearity of the calibration curve by utilizing the least square linear regression technique.

2.4.3 Preparation of Standard stock solutions:

A. Prepare of Standard stock solutions: Precisely weight 50mg of Atenolol transferred 50 ml of volumetric flasks, 50ml of diluents was added as well as sonicated for 30 minutes.
B. Prepare of Standard working solutions : 50µl of Atenolol from stock solution was pipetted out and get into a 10ml volumetric flask and invented with diluent. (50µg/ml of Atenolol).
C. Prepare of Standard Working solutions: 1ml of Atenolol taken from 50µg/ml of stock solution and add the 1ml of methanol made up the stock solution (25µg/ml).
E. Prepare of Standard Working solutions : : 1ml of Atenolol taken from 25µg/ml of stock solution and add the 1ml of methanol made up the stock solution (12.5µg/ml).
F. Prepare of Standard Working solutions: 1ml of Atenolol taken from 12.5µg/ml of stock solution and add the 1ml of methanol made up the stock solution (6.25µg/ml).
G. Prepare of Standard Working solutions: 1ml of Atenolol taken from 6.25µg/ml of stock solution and add the 1ml of methanol made up the stock solution (3.125µg/ml).
H. Prepare of Standard Working solutions: 1ml of Atenolol taken from 3.125µg/ml of stock solution and add the 1ml of methanol made up the stock solution (1.5625µg/ml).

2.3.4 LOD sample's Preparation:

About 26µl of standard stock solution (Atenolol) was pipette out as well as transferred to 10ml volumetric flasks and made up with 974µl of diluent.

2.3.5 LOQ sample's preparation:

About 81µl of standard stock solution (Atenolol) was pipetted out and transferred to 10ml volumetric flasks and made up with 919µl of diluent.

2.3.6 Accuracy:

The accurateness of the evaluate technique was find out by recovery study at three concentration levels (50.0%, 100.0%, as well as 150.0%), i.e., 85, 170, and 255 µg/ml, and three samples from every concentration were injected. The % recovery of added Atenolol and sesamol and RSD were computed for all of the replicate samples.

2.3.7 Precision:

The system accuracy and method accuracy (reliability) of the suggested approaches were evaluated through multiple measurements of a standard solution. Six measurements were taken on the same day to establish the precision of the system at the 100% concentration levels for the standard solution. The precision of the method was confirmed by conducting six separate tests on the sample solution with a concentration level of 100% on the same day. The repeatability of the obtained results was assessed by calculating the RSD..
Prepare of Standard stock solution's: : Accurate weighed 50mg of Atenolol shifted 50 ml of volumetric flasks, 50ml of diluents was added as well as sonicated for 30 minutes.

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Prepare of Standard working solutions : About 81µl of standard stock solution (Atenolol) was pipetted out and moved to 10ml volumetric flasks as well as made up with 919µl of diluent.

2.3.8 Robustness.:
Robustness of the technique was verified by apply minor and calculated changes in the investigational parameters:
Flow rates: ±0.20 mL/min
Wavelength: ±3.0 nm

Mobile's phase composition: ±2 % Acetonitrile in mobile phase

III. RESULTS AND DISCUSSION

3.1 Preliminary studies:
Determination of melting point:
The melting point of drug sample was examined by utilizing melting point apparatus. The results was as follows.

Melting point of selected drug

S.no.	Drug name	Melting point
1	Atenolol	146-148℃

3.1.1 UV-Visible Spectroscopy study:
3.1.2 Finding of λmax:
Absorption maxima (λmax) of selected drugs i.e. Atenolol was found to be 226 nm.

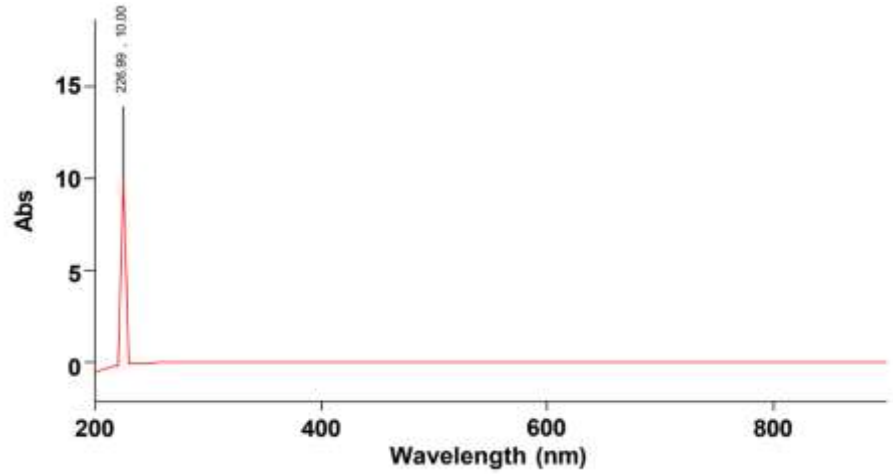

Fig. Absorption maxima (λmax)

3.2 Method Development and Validation:
3.2.1 Method development for selected drugs:
After the selection of the drugs, they were mixed in appropriate diluent to acquire a clear solution. The movable phase was improved by modify dissimilar mixtures of buffers and organic solvents. The pka value of selected drug was also considered for improving of pH of the buffer. Detection wavelength for Atenolol was taken as 276nm. The peak shape of selected drugs was establish significant with the mobile phase composition of Methanol: Water isocratic (50: 50) at a stream rate of 1ml/min. Agilent TC- C18(2), 4.6X250mm, 5 µm column with a flow rate 1ml/min and column temperature is ambient had resulted in outstanding, elution of the Atenolol drug with low retention as well as run time. The retention of Atenolol was found to be 2.010min.

(A)-METHOD OPTIMIZATION
Method-(A) (Failed method)
ACN and Water 50:50 with 0.1% TFA

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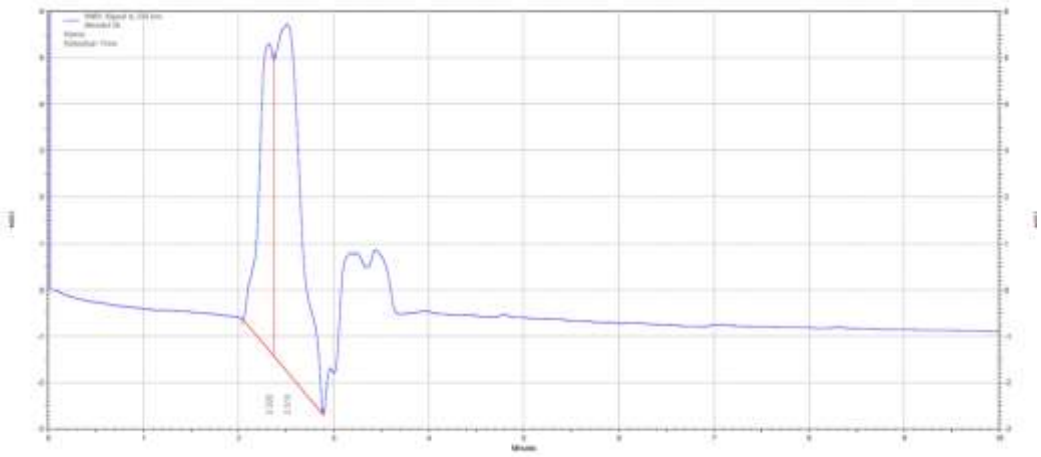

Fig: Chromatogram obtained by Method-A followed for determination of Atenolol

Method-B (Passed method)
Methanol and Water 50:50

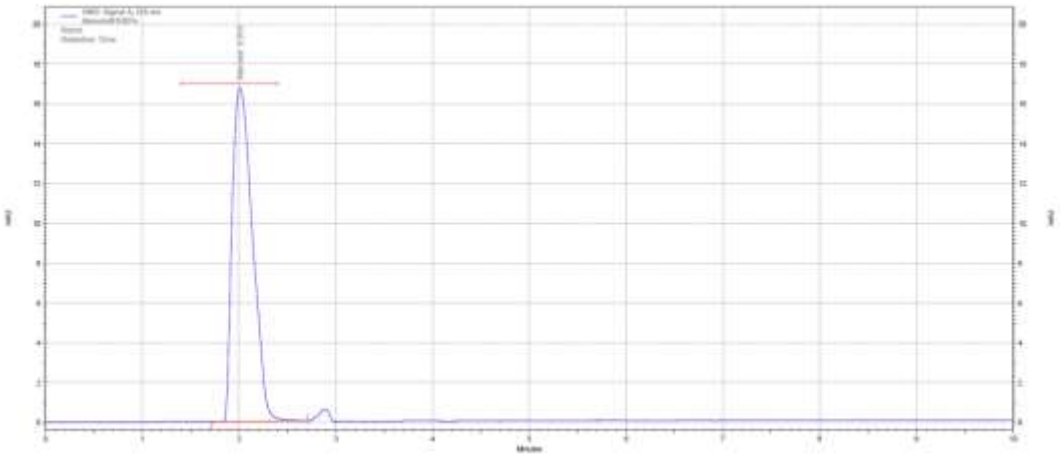

Fig: Chromatogram obtained by Method-B followed for determination of Atenolol

Prepare of Mobile Phase:
A Mixture of Methanol and Water (50:50) was prepared.
The prepared mixture was degassed in an ultra sonicator for 15 min**.**

Flow:	1.000 mL/min
Low Pressure Limit:	0.00 bar
High Pressure Limit:	400.00 bar
Maximum Flow Gradient:	100.000 mL/min²

Solvent Composition

Channel	Name 1	Used	Percent %
A	Methanol	Yes	50.0
B	Water	Yes	50.0

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Stock solution of standards (Atenolol):

Stock Solution= 50 mg in 50 ml Sonicated for 30 min Weighed accurately 50 mg of standard powder and mixed in 50 ml of HPLC grade Methanol. Stock solutions were well dissolved, sonicated for 30 minutes and filtered using 0.22µm 25mm nylon membrane filters before preparation of final working solution. Working standard solution of drugs was get ready by suitable dilution with water. Stock calibration curve was prepared from 50-1.5 µg/ml. Stock solution was diluted to obtain desired concentrations of working solutions by serial dilution method. Range of concentration used for the linearity study were 1.5625, 3.125, 6.25, 12.5, 25 and 50 ug/ml.

Chromatographic Conditions:

Stationary phase Agilent TC-C18(2),4.6x250mm,5um
Mobile's phase: Methanol: Water Mobile phase ratio: 50:50
Detection. Wavelength: 276 nm
Flow Rate: 1.0 ml/min
Sample Volume: 20µl
Temperature: Ambient
LC System: Agilent test system and OpenLab CDS2

3.3 Validation of developed methods for the estimation of Atenolol

3.3.1 Linearity:

To calculate the linearity as well as range of the technique, dissimilar standard solutions were organized by diluting the standard stock solution with acetonitrile of concentrations: 20, 10, 5, 2.5, 1.25 µg/ml. Three shots from every concentration were investigated under the similar conditions. Linear regression examination was utilized to estimate the linearity of the calibration curve by utilizing the least square linear regression technique.

S.No.	Concentration	Replicate	RT	Mean	SD	%RSD of RT	Peak Area	Mean	SD	% RSD of Peak Area
1	50	1	1.863	1.895	0.034	1.771	3258916	3252050.667	44545.563	1.370
2	50	2	1.893				3292765			
3	50	3	1.93				3204471			
4	25	1	1.883	1.884	0.002	0.123	1896129	1881913.333	12812.051	0.681
5	25	2	1.883				1878333			
6	25	3	1.887				1871258			
7	12.5	1	1.88	1.857	0.047	2.510	912057	936179.333	21653.825	2.313
8	12.5	2	1.803				942542			
9	12.5	3	1.887				953939			
10	6.25	1	1.69	1.689	0.005	0.304	614780	618587.667	5750.824	0.930
11	6.25	2	1.693				625203			
12	6.25	3	1.683				615780			
13	3.125	1	1.683	1.687	0.004	0.208	281620	284310.333	3340.965	1.175
14	3.125	2	1.687				283261			
15	3.125	3	1.69				288050			
16	1.5625	1	1.667	1.692	0.022	1.302	150335	152145.333	1738.601	1.143
17	1.5625	2	1.703				152299			
18	1.5625	3	1.707				153802			

Calibration Graphs of Atenolol

S.No	Concentration in (µg/ml)	Area (Amu)
1	25	2013107
2	12.5	1111865
3	6.25	594780
4	3.125	281620
5	1.5625	150335

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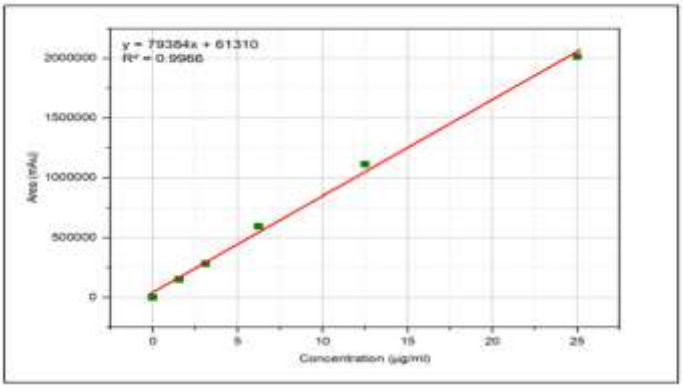


Fig.4.7 Calibration graph of Atenolol

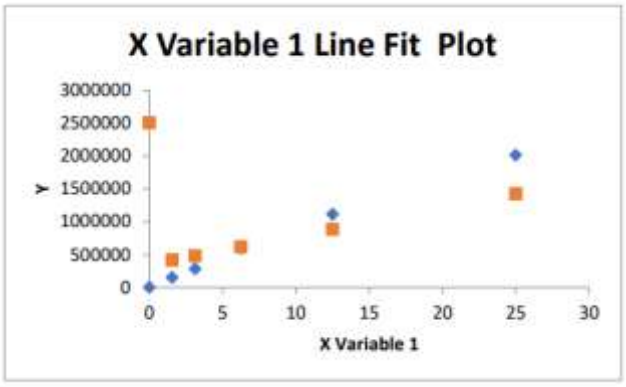
3.3.2 LOD AND LOQ determination of Atenolol:

SUMMARY
OUTPUT

Regression Statistics	
Multiple R	0.91601175
R Square	0.83907752
Adjusted R Square	0.7988469
Standard Error	391488.59
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.1966E+12	3.1966E+12	20.8566883	0.010284813
Residual	4	6.1305E+11	1.5326E+11		
Total	5	3.8096E+12			



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	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	347091.95	222626.961	1.55907419	0.19398629	-271019.587	965203.488	-271019.587	965203.488
X Variable 1	43140.9503	9446.41524	4.56691234	0.01028481	16913.49697	69368.4037	16913.49697	69368.4037
	Slope							

RESIDUAL OUTPUT

LOD	26.78479549
LOQ	81.16604695

Observation	Predicted Y	Residuals
1	2504139.47	-326607.467
2	1425615.71	587491.291
3	886353.829	225511.171
4	616722.89	-21942.8898
5	481907.42	-200287.42
6	414499.685	-264164.685

SD	350138.04

3.3.3 Precision:

The precision of the developed technique was measured for intraday (Precision) and inter-day (intermediate precision). The obtained % RSD for Atenolol was 0.050 for Intraday and 1.645 for inter-day respectively.

[A] Repeatability at LOQ of Atenolol:

Atenolol

S.No	Sample Id	RT	Peak Area
1	Rep-I	2.01	4376381
2	Rep-II	2.017	4448479
3	Rep-III	2.02	4415735
4	Rep-IV	2.02	4327475
5	Rep-V	2.08	4374289
6	Rep-VI	2.017	4501340
7	mean	2.0273333	4407283.167
8	stdev	0.0260589	61689.74059
9	**%RSD**	**1.2853785**	**1.399722647**

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[B] Interday:

S.No	Day	Concentration(µg/ml)	Replicate-1	RT	Mean	SD	%RDS	Mean of RDS of RT	Peak Area	Mean	SD	%RDS	Mean % RDS of Peak Area
1	1		1	2.02	2.0156667	0.0051316	0.2545858		4415735	4413532	36099.465	0.818	
2			2	2.017					4448479				
3			3	2.01					4376381				
4	2		1	2.07	2.0366667	0.0305505	1.5000248		4030754	4196411	143526.064	3.420	
5			2	2.03					4283488				
6			3	2.01					4274991				1.645
7	3		1	2.02	2.016	0.0034641	0.1718304		4030754	4063078	28313.677	0.697	
8			2	2.014				0.64214701	4083488				
9			3	2.014					4074991				

[C] Intraday

S. No	Time	Conc.(µg/ml)	Replicate-I	RT	Mean	SD	%RDS	Mean % RSD of RT	Peak Area	Mean	SD	%RDS	Mean % RSD of Peak area
1	1st hours		1	2.017	2.039	0.0355387	1.7429481	0.762731738	4501340	4634368.000	390775.030	0.084	0.050
2			2	2.08					5074289				
3			3	2.02					4327475				
4	2nd hours		1	2.02	2.0156667	0.0051316	0.2545858		4415735	4313531.667	206030.026	0.048	
5			2	2.017					4448479				
6			3	2.01					4076381				
7	3rd hours		1	1.993	1.9863333	0.0057735	0.2906613		2871345	2843346.000	51142.192	0.018	
8			2	1.983					2784318				
9			3	1.983					2874375				

3.3.4 Recovery:

S.No	Concentration(µg/ml)	%Skiped Level	Peak Area	Recovery	Mean Recovery
1	LOQ	100% sample	4471706	97.459	
2		80% 0.75 ug/ml std	3277364.8		
3		100% sample+80% 0.75 ug/ml std	7951124.8		
		Expected value	7749070.8		
1	LOQX2	100% sample	8145307	98.660	101.928
2		80% 0.75 ug/ml std	6215115.6		
3		100% sample+80% 0.75 ug/ml std	14555417.6		
		Expected value	14360422.6		
1	LOQX4	100% sample	13645217	109.664	
2		80% 0.75 ug/ml std	10311241		
3		100% sample+80% 0.75 ug/ml std	21845231		
		Expected value	23956458		

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3.3.5 Robustness:

S.No	Parameter	Condition	RT	Mean	SD	%RDS	Mean %RSD of RT	Peak Area	Mean	SD	%RDS	Mean %RSD of RT
1	Flow rate	0.8	1.917	1.947	0.055	2.819	1.425	4802109	4724982	91343.1	1.933	2.728
2		0.8	1.913					4815223				
3		0.8	2.01					4711104				
4		1.2	2.04	2.039	0.019	0.908		4557762	4558134	176490	3.872	
5		1.2	2.057					4790201				
6		1.2	2.02					4732668				
7		1	2.07	2.057	0.011	0.548		4665807	4440859	105682	2.380	
8		1	2.05					4392879				
9		1	2.051					4421849				
10	Mobile Phase	MeOH-48	1.913	1.865	0.047	2.500	1.560	4345769	4380044	44261.8	1.011	2.072
11		MeOH-48	1.861					4441665				
12		MeOH-48	1.82					4445608				
13		MeOH-50	2.057	2.050	0.008	0.399		4372434	4327260	76505	1.768	
14		MeOH-50	2.041					4352379				
15		MeOH-50	2.052					4351378				
16		MeOH-52	2.093	2.058	0.037	1.780		4351077	4315715	148416	3.439	
17		MeOH-52	2.02					4341066				
18		MeOH-52	2.062					4367052				
19	Wave length	274 nm	2.477	2.473	0.004	0.153		4155436	4386140	191602	4.368	
20		274 nm	2.47					41504				

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								33				
21		274 nm	2.471					4261486				3.012
22		276 nm	2.087	2.069	0.025	1.226	1.393	4583458	4485652	102017	2.274	
23		276 nm	2.04					4484234				
24		276 nm	2.08					4576888				
25		278 nm	2.547	2.486	0.070	2.801		4491047	4423111	105857	2.393	
26		278 nm	2.41					4477144				
27		278 nm	2.5					4301141				

Atenolol-Robustness was performed with variation in 3 parameter-

(1) wavelength (±3 nm),
(2) flow rate (±0.2min),
(3) mobile phase concentration (±2% organic solvent)

IV. CONCLUSION

A detailed literature survey revealed that selected drugs i.e Atenolol have been successfully identified and validated by using HPLC. However, their estimations revealed higher retention times as well as inclusion of costly chemicals. Thus, the present researcher conceptualized the "Developments as well as Validation of new investigative techniques for estimation of Antihypertensive drug. Analysis of the selected drug in their standard and bulk samples has been successfully accomplish by the application of the developed RP-HPLC method. Drug peaks were well developed by using appropriate mobile phase system.

Preliminary studies conducted on the chosen drug i.e. Atenolol comprised of UV-Visible spectroscopic studies. Absorption maxima (λmax) of selected drugs i.e. Atenolol was found to be 226nm.

Specificity try-out was done by injecting samples of the movable phase, placebo, sample solution, un-spiked and spiked sample. The end results showed no interfering at the retention time of Atenolol.

Standard solutions of Atenolol (50ppm-1.5625ppm) was prepared and injected. Calibration curves was drawn and the concentration of drug versus corresponding peak areas find at 276nm.

The drug showed a linear response. In the present work, LOD of Atenolol was determined by linearity curve method. LOD values was found to be 26.78479549.

The percent mean recovery for Atenolol was 101.928, indicating that the method was accurate.

The precision of the developed technique was measured for intraday (Precision) and inter-day (intermediate precision). The obtained % RSD for Atenolol was 0.050 for Intraday and 1.645 for inter-day respectively.

Robustness was carry out by making slight dissimilarity in the Flow rate (flow minus &flow plus), column temperature (Temperature minus & Temperature Plus) and concentration of the mobile phase (Mobile phase minus & Mobile phase plus). The %RSD of the above conditions were calculated. No significant outcome was find with the above changes representing the robustness of the technique.

Hence, it was concluded that the techniques adopted for the evaluation of Atenolol was simple, precise accurate and stable. The technique was originate to have a appropriate application in routine lab examination with a high degree of accurateness and precision.

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