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METHOD DEVELOPMENT AND VALIDATION OF AN ANTI-HYPERTENSIVE AGENT BY USING RP-HPLC

Badikela Ramakrishna*, Kondapuram Parameshwar, Donapati Jalander and Kosika Sandeep

Gurunanak Institute of Technical Campus (School of Pharmacy).

***Corresponding Author: Badikela Ramakrishna**
Gurunanak Institute of Technical Campus (School of Pharmacy).

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ABSTRACT
A new Simple, rapid and sensitive isocratic RP-HPLC method has been developed and validated for the determination of Atenolol in tablet formulation. The method employs SHIMADZU HPLC system on ODS, C18 column (250*4.6mm and 5 µm). Best chromatographic separation was achieved by using 30mm Ammonium acetate (pH 3): acetonitrile: methanol in the ratio of 50:30:20 as mobile phase with a flow rate of 1.0mL/min and isocratic elution with a total runtime of 5 minutes. Detection of the compound was carried out at 226nm. The retention time of Atenolol was found to be 2.527. The linearity studies ranges from at the concentration range of 60-140µg/ml. This method was validated for accuracy, precision, linearity, ruggedness and robustness as per ICH guidelines. The present newly developed method was found to be accurate, precise and can be useful for routine Quality control analysis.

KEYWORDS: Atenolol, HPLC, ODS, ICH.

INTRODUCTION
Chromatography may be defined as a non destructive procedure for separating mixture of components into individual components through equilibrium distribution between two phases

The technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium under the influence of solvent[1] or gas.

High Performance Liquid Chromatography (Hplc)
It has also been referred to as High Pressure LC)
HPLC is a separation technique that involves:
• The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (µm) in diameter called the stationary phase where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.[2]
• These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount[3]. An output from this detector is called a "liquid chromatogram".

Reverse Phase HPLC
Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.[4]

The principle involved in HPLC is adsorption. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase. The solute molecules partition (i.e. an equilibrium is established) between the mobile phase and the stationary phase. The distribution of the solute between the two phases depends on the binding properties of the medium[5], the hydrophobicity of the solute and the composition of the mobile phase.

Chemically bounded octadecyl silane (ODS) and alkane with 18 carbon atom is the most popular stationary phase used in pharmaceutical industry. Organic solvents water and buffers are used as mobile phase.[6]

HPLC is a method of choice in the field of analytical chemistry since the method is specific, robust, linear, precise and accurate; LOD is low and also offers

1. Speed (many analysis may be accomplished in 20min)
2. Greater sensitivity (various detectors can be employed)
3. Improved resolution
4. Reusable column
5. Ideal for substance of low viscosity
6. Easy sample recovery
7. Precise and reproducible

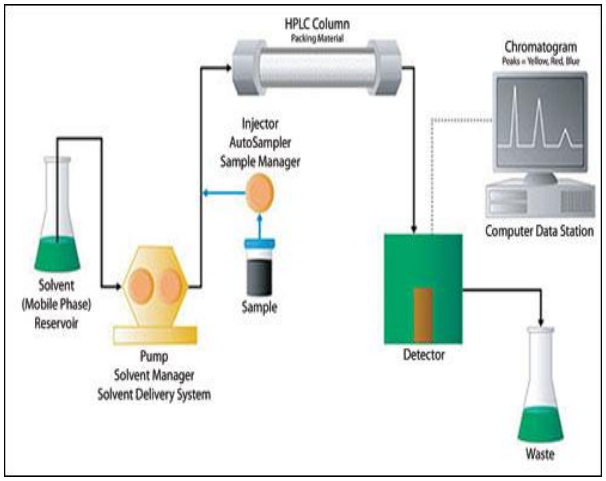


Fig. 1: Schematic representation of HPLC system.

MATERIALS AND METHODS

Materials

Atenolol (purity: 98.91%), Saimira (Chennai, India) and Micro labs (Bangalore, India) respectively. Acetonitrile was of HPLC grade and obtained from E. merck (Mumbai, India) andall other chemicals used were of analyticalgrade. Purified water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis

Method Development

Prior to the initiation of method development, all the information about the analyte such as its structure, physical and chemical properties toxicity, purity, hygroscopicity, solubility and stability should be determined. The goals and requirements of the hplc method that needs to be developed should be known as well as the analytical figures of merit,[7] which include the required detection limits selectivity, range, accuracy and precision.

Method Development Considerations

Sample Properties

Analyte structure and pka: If the target substance is ionizable, the pka of the analyte should be determined. The optimal pH, to commence method development, is at a pH that is atleast 1- 2 units from the analyte pka in the particular hydro-organic mixture that is employed.

Solubility of components: solubility of components is also very important. The analyte must be in the diluent and must not react with any of the diluent components. the diluent should match to the starting eluent composition of the essay to ensure that no peak disortion will occur, especially for early eluting components.

Detector Consideration

Choice of the proper detection scheme is dependent on the properties of the analyte. Different types of detectors are available such as ultraviolet(UV), fluorescence, electrochemical, light scattering, refractive index(RI), flame ionization (FID), evapourating light scattering detection (ELSD), corona aerosol detection(CAD), mass spectrophotometric (MS), NMR, and others. However the majority of reversed phase and normal phase HPLC method development in the pharmaceutical industry is carried out with UV detection. A wavelength for UV detection must be chosen so that an accurate mass balance may be determined. Therefore if area % normalization is to be used then all the impurities and the active pharmaceutical ingredient must have similar relative response factors (area (Page: 16) response /weight). The UV spectra of target analyte and impurities must be taken and overlaid with each other, and the spectra should be normalized due to different amounts present in the mixture.[8-10]

A wavelength must be chosen such that adequate response is obtained for the active and that at least a 0.05 v/v% solution of the active at target concentration should be quantified (S/N greater than10). The wavelength chosen should not be distinct slope of the spectrum, and the relative difference in the absorbance at a certain wavelength is not significantly different from the impurities/degradation products present.[11]

Mobile Phase Parameters

Organic Solvent Strzength and Selectivity

Solvent strength or % organic solvent content in the mobile phase controls the retention time of analyte and that different organic solvents (MeOH, ACN, THF) can have a dramatic effect on selectivity. In the development of reverse phase separation methods the organic part of the eluent is considered the strong solvent. Organic solvent increases the solvent strength and allows for elution of the species in the mixture, resulting in smaller analyte retention factors or retention volumes. Increasing the concentration of organic modifier generally leads to an exponential increase in the analyte retention volume. The general rule of thumb is that for every 10v/v% increase in organic modifier there is two to three fold decrease in the analyte retention factors or analytes with molecular weights of less than 1000Da. Increasing the fraction of mobile phase strength depends not only on the concentration of the organic modifier, but also on the type of organic modifier used. The solvent strength of the most common organic eluents used at the same volume percentage (v/v%) in the reversed-phase chromatography would be methanol < Acetonitryl < Tetrahydrofuran

Buffers: Many drugs have either acidic or basic functional groups and can exist in solutions in ionized or

unionized forms. The ionic state and the degree of ionization greatly affect their chromatographic retention in RPLC. Typically, the ionic form does not partition well into the hydrophobic stationary phase and therefore has significantly lower k` than the neutal, un-ionized form. Buffers are commonly used to control the pH of the mobile phase for the separation of acidic or basic analytes.[12-14]

Volatile acids and their ammonium salts are used for (the development of mass spectrometer (MS) compatible HPLC methods. since a buffer is only effective within 1-2 ph unit from its pka judicious selection of the proper buffer with in its buffering range is paramount.

Acidic Mobile Phase

A mobile phase at acidic pH of 2.5-3 is a good starting point or most pharmaceutical applications because the low pH suppresses the ionization of most acidic analytes resulting in their higher retention. Common acids used for mobile phase preperations are phosphoric acid, formic acid and acidic acid. Low pH also minimizes the interaction of basic analytes with surface silanols on the silica packing (because silanol do not ionize at acidic pH) also, the lifetime of the silica based clumns is excellent in the pH range of 2.5. However the basic analytes are ionized at low pH and might not be retained unless ion pairing reagents are used.

Ion Pairing Reagents

Ion pairing reagents are detergent like molecules added to the mobile phase to provide additional retention or selectivity for the analytes with opposite charge. Long chain alkyl sulphonates are commonly used for water soluble basic analytes. Amine modifiers such as tetra ethyl amine (TEA) are often used in the mobile phase to reduce peak tailing caused by the strong interaction of basic analytes with acidic surface silanols. For acidic analytes, ion pairing reagents such as tetra alkyl ammonium salts are often used.

High pH Mobile Phase

The advent of silica based columns stable under high pH conditions offers an important alternate approach for the separation of basic analytes. The application of this approach is increasing for assay and impurity testing of many water soluble basic drugs. The advantage of high pH separation as opposed to ion-pairing is mass spectrometry compatibility, better sensitivity and unique selectivity.

Isocratic VS Gradient Analysis

Traditionally, most pharmaceutical assays are isocratic analysis employing the same mobile phase throughout the elution of the sample. Isocratic analyses are particularly common in quality control application since they use simpler HPLC equipment and premixed mobile phases. Notable disadvantage of isocratic analysis are limited peak capacity (the maximum number of peaks that are obtained in the chromatogram) and problems with samples containing analytes of diverse polarities. also, late eluters (such as dimmers) are particularly difficult to quantitative in isocratic analysis due to excessive band broadening with long retention times.

In contrast the gradient analysis in which the strength of mobile phase is increased with time during sample elution, is suited for complex samples and those containing analytes of wide polarities. Gradient chromatography is amenable have high throughput screening applications and for impurity testing. The disadvantage of gradient analysis is the requirements for more complex instrumentation and greater skills in method development and difficulties in method transfer.[15]

Stationary Phases for Reverse Phase HPLC

Silica based packing materials dominate in application for RP separations in pharmaceutical industries. Hydrophobic surface of these packings typically are made by covalent bonding of organosilanes on the silica surface. This modification involves the reaction on monofunctional alkyl dimethylchlorosilanes with the surface silanol groups. Octadecylsilane was the first commercially available silica based bonded phase and is still the most commonly utilized. Also alkyl type ligands of different number of carbon atoms (C1, C4, C8, and C12) are often used as well as phases with phenyl functionality. Polar embedded phases provide an additional avenue for potential modification of the chromatographic selectivity, and some of these phases offer an enhancement of retention of polar analytes. These phases can be used with high aqueous mobile phases, even 100% aqueous, without loss of analyte retention that sometimes could be observedfor more hydrophobic forces. Mosed silica- based reversed-phase packing materials have a relatively narrow applicable pH range.[16-17]

Method Validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical method validation is the process of demonstrating that an analytical method is reliable and adequate for its intended purpose. Any method that is utilized to determine results during drug substance and formulation development will have to be validated.

Validation of HPLC method focuses on the following:

- Identification tests
- Quantitative measurements of the content of related substance
- Quantitative and limit tests for the control of related substance
- Quantitative tests for the assay of major components (eg: drug substance and preservatives) in sample of drug substance or drug product (assay, content uniformity, dissolution rate, etc)

Analytical method validation is established through documented evidence demonstrating the accuracy, precision, linearity, selectivity, ruggedness, and robustness of that particular test method which will be utilized to generate test results for a drug substance or drug product.

Parameters for the Method Validation

Specificity/Selectivity

The term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method, which provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from each other if the response is distinguished from all other responses the method is said to be selective

Precision and Reproducibility

Precision of a method is the extent to which individual test results of multiple injections of a series of standards agree the measured standard deviation can be sub divided into three categories:

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. it is also termed as intra-day precision.

Intermediate precision

Intermediate precision expresses within laboratories variations: different day, different analyst, different equipments etc

Reproducibility

Reproducibility expresses the precision between laboratories collaborative studies, usually supplied to standardization of methodology.

Ruggedness

The precision obtained when the assay is performed by multiple analysis, using multiple instruments, on multiple days, in one laboratory, different sources of reagents and multiple lots of columns should also be included in this study.

Accuracy

The test for accuracy is intended to demonstrate the closeness of agreement between the value found and the value that is accepted either as conventional true value or as an accepted reference value. Thus the accuracy of the method is the closeness of the measured value to the true value for the sample. The accuracy can also be determined by recovery of the impurity spiked to a drug substance or into placebo with drug substance. The percentage recovery with the certain acceptance criteria at each defined level is reported. Accuracy should be assessed using a minimum of nine determination at a minimum of three concentration levels covering the specified range.

Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. It is recommended to perform the linearity of the API and related substances independently and once linearity has demonstrated, linearity could be performed containing both API and specific related substance if necessary. At least five concentrations within the range specified above for linearity test should be used.

Limit of Detection

LOD is defined as lowest concentration of the analyte that can be detected, but not necessarily quantified, by the analytical method.

Based on visual evaluation: The detection limit is determined by the analysis of samples with known concentrations of the analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on signal to noise ratio: A signal to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit

Based on standard deviation of the response and slope

The limit of detection (LOD) may be expressed as

$$\mathbf{LOD = 3.3\ \sigma/S}$$

Where σ = the standard deviation of the response

S = slope of calibration curve of analyte

Limit of Quantification

LOQ is defined as the lowest concentration of the analyte that can be determined with acceptable accuracy and precision by the analytical method.

Based on visual evaluation: the quantization limit is determined by the analysis of the samples with known concentration of analyte

Based on signal to noise ratio: signal to noise ratio between 10:1 is generally considered

Based on standard deviation of the response and slope

The limit of detection (LOD) may be expressed as

$$\mathbf{LOD = 10\ \sigma/S}$$

Where σ = the standard deviation of the response

S = slope of calibration curve of analyte

Robustness

Robustness tests examine the effect operational parameters have on the analysis results.

Range
The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

System Suitability Parameters
The purpose of the system suitability test is to ensure that the complete testing system (including instrument, regents, columns, and analysts) is suitable for intended application.

After the method has been validated an overall system suitability test should be routinely run to determine if the operating system is performing properly.

Commonly used system suitability parameters are as follows:-
Retention Time (RT): Retention time is the time of elution of peak of maximum after injection of compound.

Theoritical Plates (N)
It is also called as column efficiency. A column can be considered as being made of large number of theoretical plates where distribution of sample between liquid-liquid or solid-solid phase occurs. The number of theoretical plates in the column is given by the relationship

$$N = 16(t/w)^2$$

Where t is the retention time and w is the width at the base of the peak.

$$HETP = L/N$$

Where L is length of the column.

Theoretical plates should be more than 2000.

Resolution (R)
It is the function of column efficiency and is specific to ensure that closely eluting compounds are resolved from each other to establish the general resolving power of the system.

For the separation of two components in the mixture the resolution is determined by equation.

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}$$

Where t_1 and t_2 are the retention time of second and first compounds respectively, where as W_1 and W_2 are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines.

R should be more than 2 between peak of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard)

Tailing Factor (T)
It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

$$R = \frac{W_{0.05}}{2F}$$

Where $W_{0.05}$ is the width of peak at 5% height and F is the distance from the peak maximum to the leading edge of the peak height from the baseline. Tailing factor should be less than 2.

Capacity Factor (K')
It is calculated by the formula.

$$K' = t/t_a - 1$$

Where tis the retention time of the drug t_a is the retention time of non retarded component, air with thermal conductivity detection.

Selectivity
Also known as separation factor, it is measure of peak spacing and expressed as.
Selectivity = k''_2 / k'_1

System suitability parameters and their standard values.

Parameter	Limit
Capacity factor	k'>2
Injection precision	RSD < 1% for n≥5
Resolution	Rs > 2
Tailing factor	T ≤ 2
Theoretical plate	N>2000

Method Validation
"A Documented Programme", which provides a high degree of assurance that a specific process will consistently produce, a product meeting its pre-determined specifications and quality attributes.

Parameters used for assay validation
The validation of assay procedure was carried out using the following parameters

- System suitability
- Linearity
- Accuracy
- Precision
- Limit of detection
- Limit of quantification
- Ruggedness
- robustness

Optimized Chromatographic Conditions
A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Atenolol in Tablet dosage form. A Thermo ODS C3 2mm column having 250 x 4.6mm id in Isocratic mode with mobile phase containing 0.1M Ammonium Acetate: ACN: methanol (50:30:20 %v/v pH3) was used. The

flow rate was 1.2ml/min and effluents were monitored at 226nm. The retention time of Atenolol was 1.2 min respectively.

MATERIAL AND METHODS

Apparatus

Waters e2695Alliance RT- HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

Other Apparatus: Electronic balance, Sonicator, 0.45µ membrane filter and UV.

Reagents and Chemicals

Pharmaceutical grade Atenolol were kindly supplied as a gift sample by Chandra Labs, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Disodium hydrogen orthophosphate were analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.

Preparation and Selection of Mobile Phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of Ammonium Acetate buffer pH 3 and Methanol and ACN were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be Buffer: Methanol (50:20 v/v) and filtered through 0.45µ membrane filter.

Preparation of Standard Solution

25mg Atenolol was dissolve in 25 ml of **(**Methanol) and mobile phase and was further diluted to get stock solution of (47.5□µg/ml). This is taken as a 100% concentration. Solution containing mixture of Atenolol of five different concentrations (50%, 75%, 100% 125%, and 150% of target concentration) were prepared in the same way.

Preparation of Sample Solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent Methanol K2HPo4 ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to one tablet weight were weighed and taken in a 25 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 15 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions

The mobile phase, a mixture of Ammonium Acetate buffer ACN and methanol (50:30:20v/v) pumped at a flow rate of 1.0 ml/min through the column (C3; 5µ, 4.6 X 250 mm, ODS) at 40ºC.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 226 nm, which was selected as wavelength for further analysis.

Development and Validation of HPLC Method

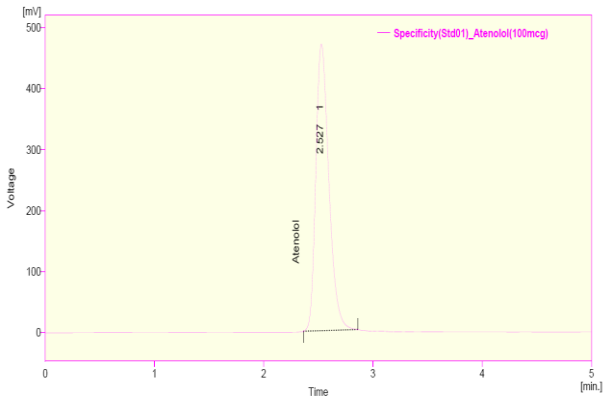
Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Atenolol in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2013.The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, LOD, LOQ, and robustness.

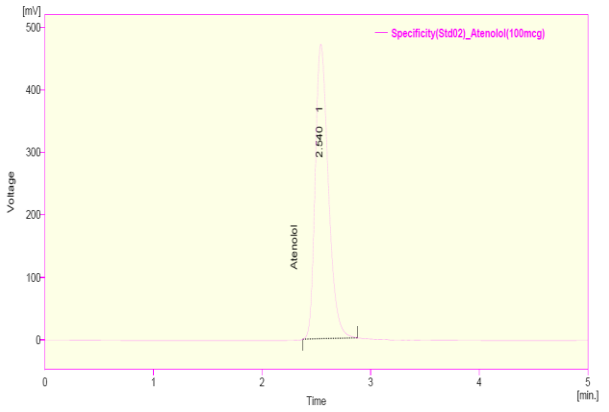
System Suitability

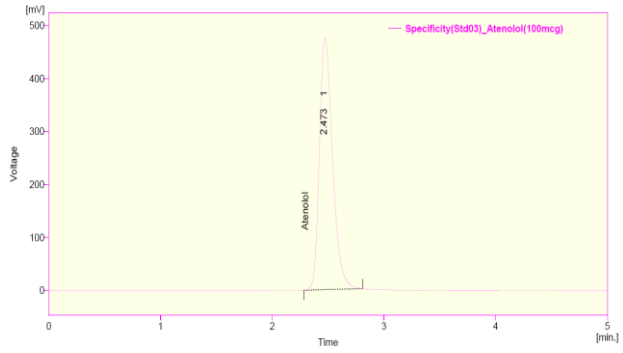
System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Atenolol. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

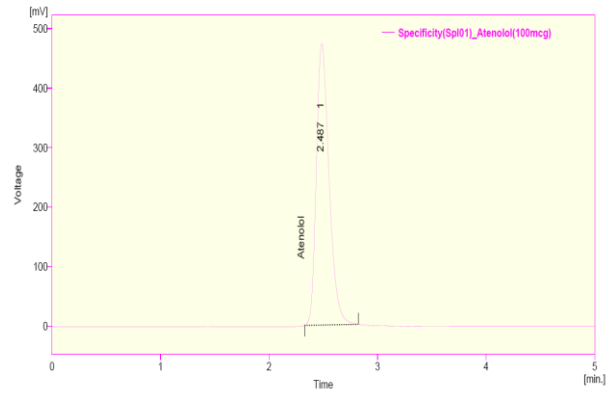
Selectivity

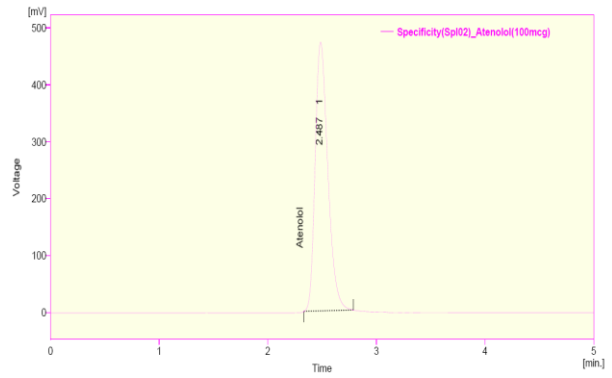
Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of Atenolol were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

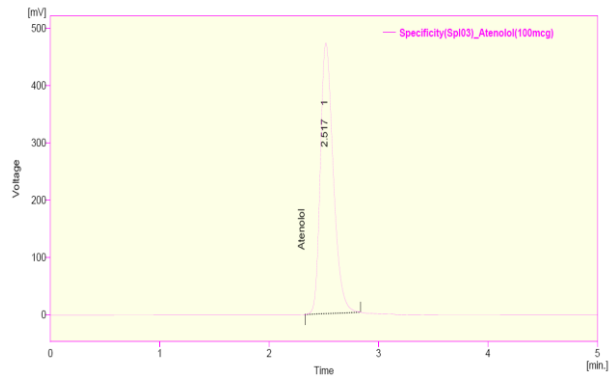












LINEARITY

Linearity of the method was determined by constructing calibration curves. Standard solutions of Atenolol of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

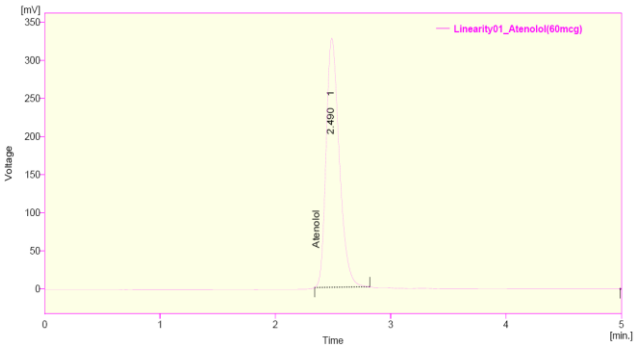


Fig: 01_Linearity01_Atenolol (60mcg).

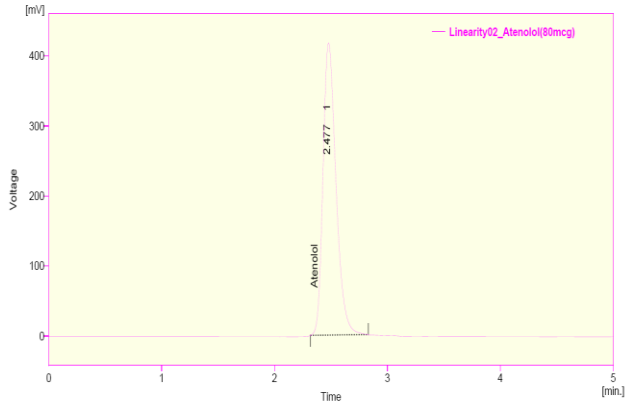


Fig: 02_Linearity02_Atenolol (80mcg).

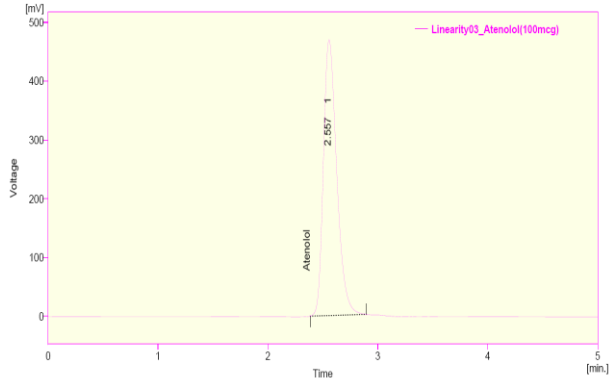


Fig: 03_Linearity03_Atenolol (100mcg).

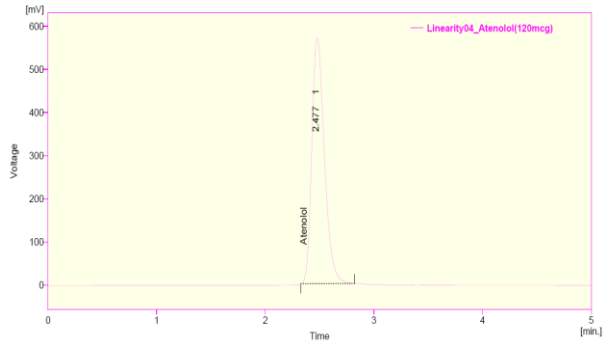


Fig: 04_Linearity04_Atenolol (120mcg).

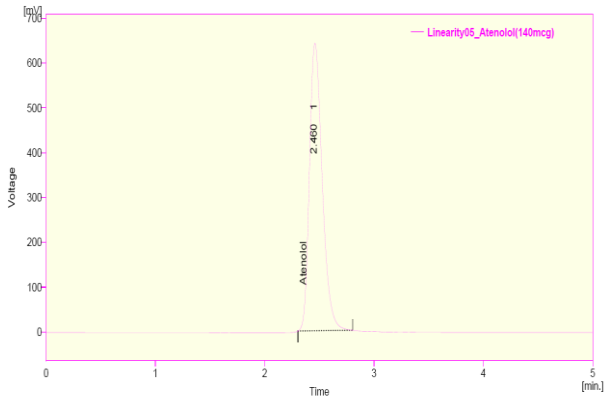
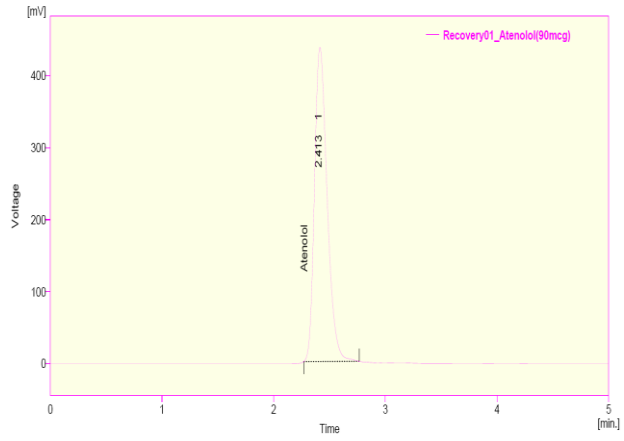


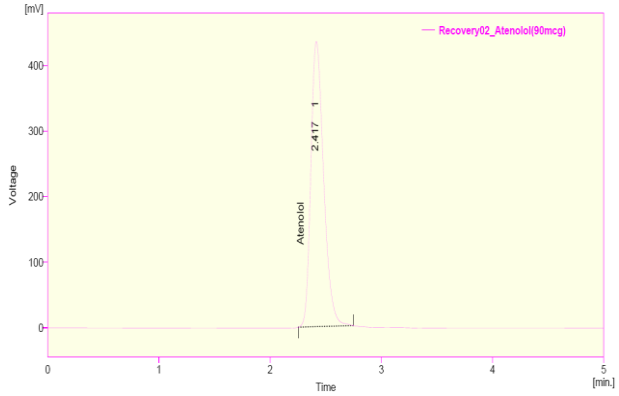
Fig: 05_Linearity05_Atenolol (140mcg).

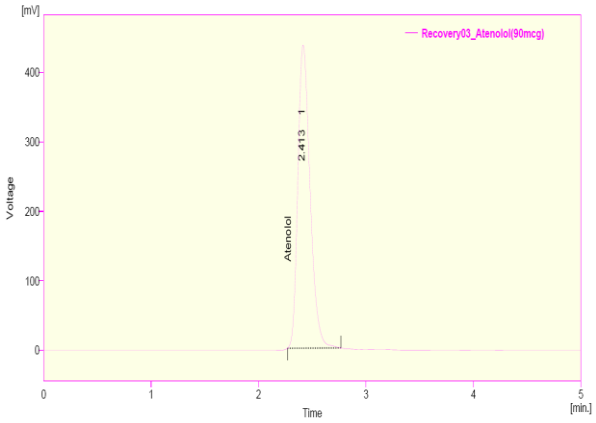
S. No	Rt	Area	Tailing	Theoritical Plates
Linearity01	2.490	2601.563	1.536	2258
Linearity02	2.477	3340.869	1.536	2234
Linearity03	2.557	3950.388	1.552	2037
Linearity04	2.477	4580.602	1.536	2118
Linearity05	2.460	5222.845	1.483	2090

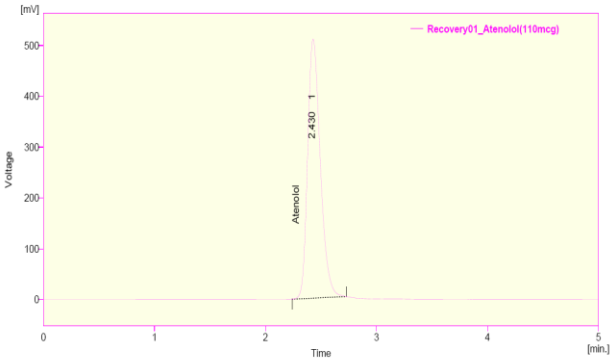
Accuracy (Recovery Studies)

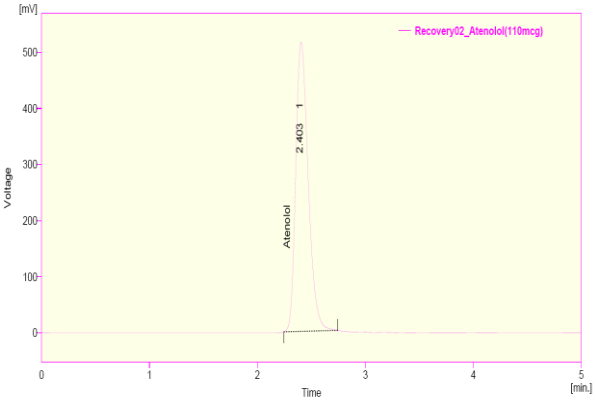
To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Atenolol were added to pre-analyzed samples and were subjected to the proposed HPLC method.

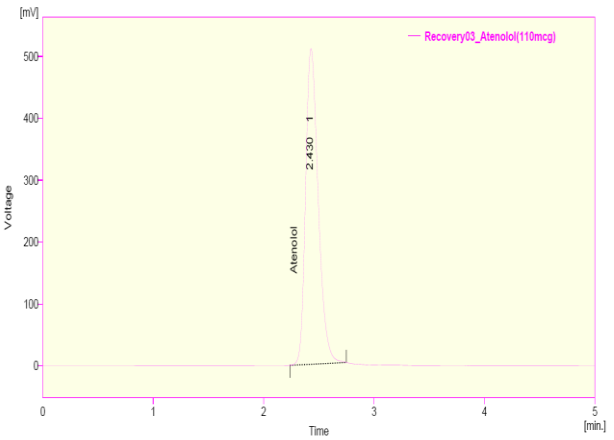


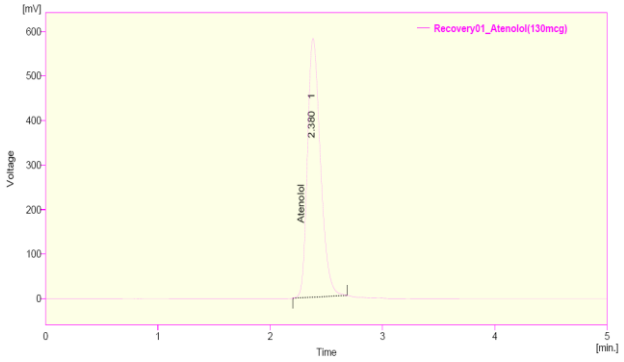


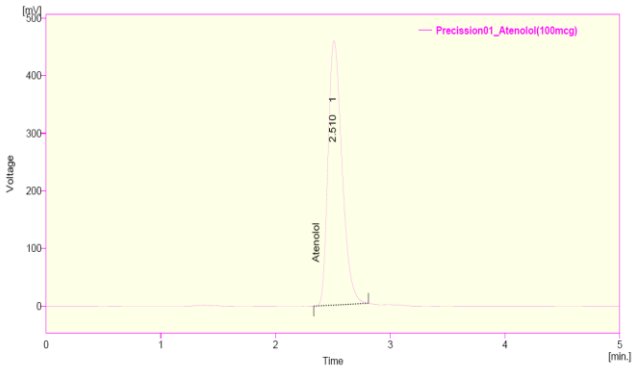


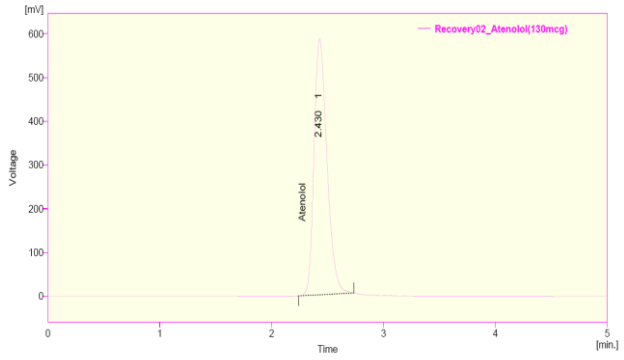


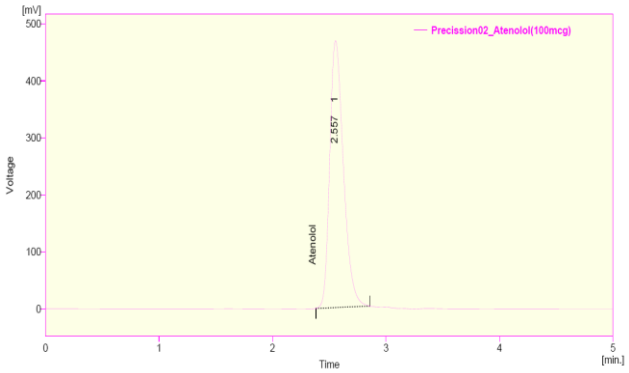


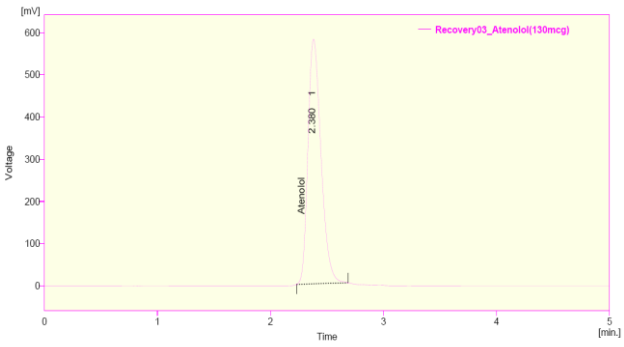


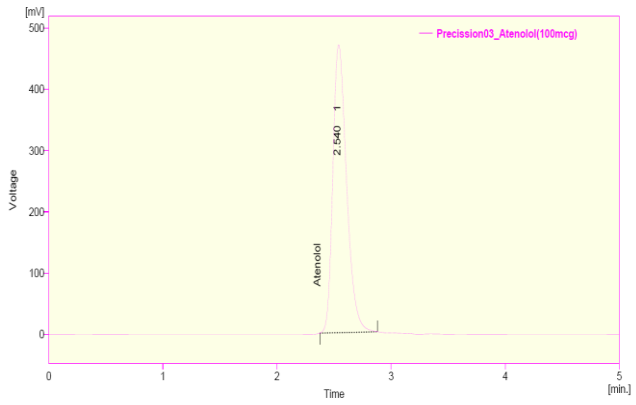


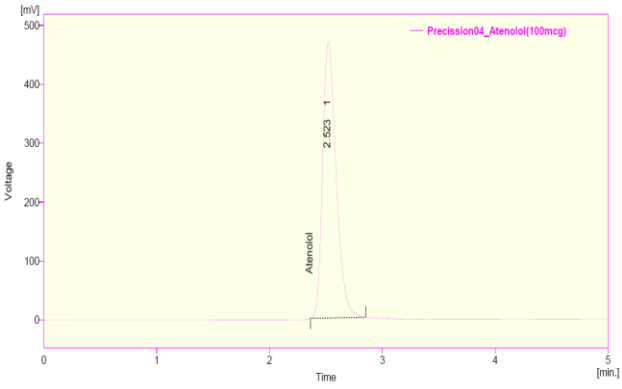








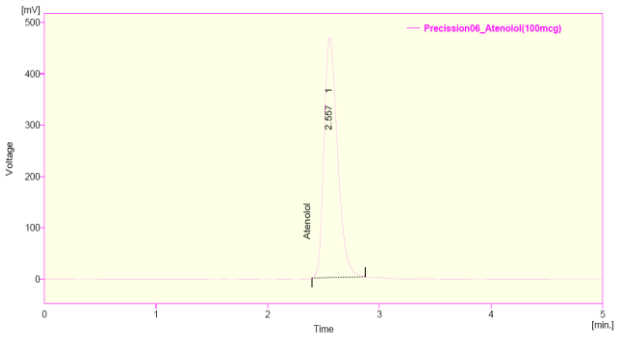




S. No.	Rt	Area	Tailing	Theoritical Plates
1.	2.413	3733.995	1.557	2081
	2.417	3767.814	1.500	2017
	2.413	3734.599	1.556	2121
2.	2.430	4333.995	1.500	2039
	2.403	4332.753	1.536	2104
	2.430	4351.045	1.500	2039
3.	2.380	4892.032	1.536	1956
	2.430	4899.228	1.536	2039
	2.38	4929.946	1.593	1956

Precision

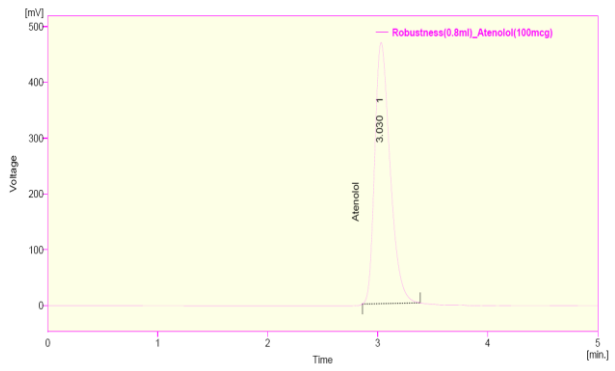
Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

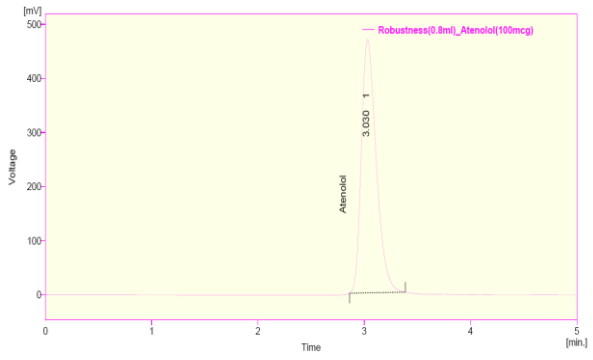


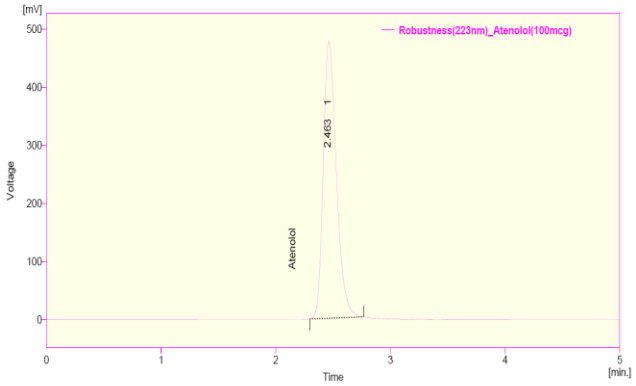
S. No	Rt	Area	Tailing	Theoritical Plates
Precision01	2.510	3883.038	1.552	1869
Precision02	2.557	3927.143	1.552	2037
Precision03	2.540	3938.118	1.552	2115
Precision04	2.523	3899.237	1.517	2087
Precision05	2.487	3838.744	1.571	2135
Precision06	2.557	3922.837	1.552	2037

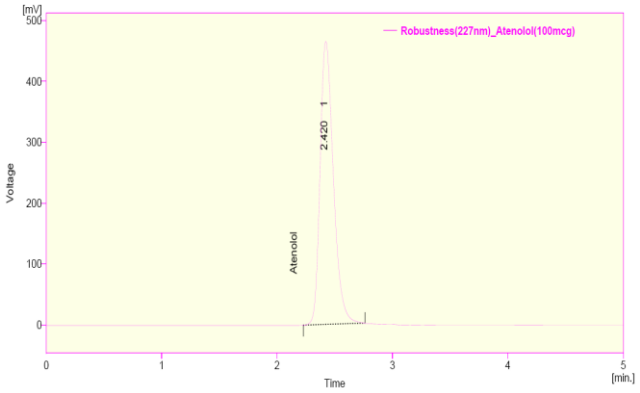
Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature.







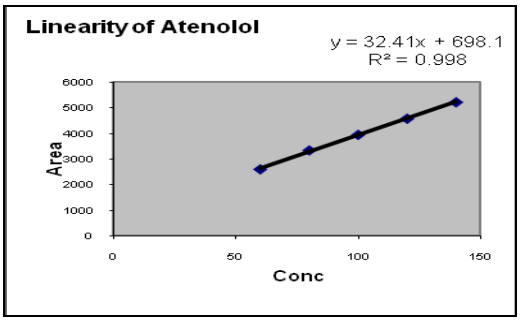


RESULTS AND DISCUSSION

Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor 0.

Linearity

ppm	Area
60	2601.563
80	3340.869
100	3950.388
120	4580.602
140	5222.845



Discussion: The method showed a linearity range from 60mcg to 140mcg.

Precision

Method Precision		
Atenolol		
S.No.	Rt	Area
1	2.51	3883.038
2	2.557	3927.143
3	2.54	3938.118
4	2.523	3899.237
5	2.487	3838.744
6	2.557	3922.837
avg	2.5290	3901.52
stdev	0.0277	36.710
%RSD	**1.10**	**0.94**

Discussion

The method shown a RSD less than 2.0% for retention time and area

Accuracy

Atenolol			
80		3340.869	
100		3950.388	
120		4580.602	
80+10	**mcg**	**90**	**mcg**
		3734.599	
		3767.814	
		3734.599	
	Avrg	**3745.671**	
result		**89.69**	**mcg**
%rec		**99.66**	**%**
100+10	**mcg**	**110**	**mcg**
		4333.995	
		4332.753	
		4351.045	
	Avrg	**4339.264**	
result		**109.84**	**mcg**
%rec		**99.86**	**%**
120+10	**mcg**	**130**	**mcg**
		4892.032	
		4899.228	
		4929.946	
	Avrg	**4907.069**	
result		**128.55**	**mcg**
%rec		**98.89**	**%**

Discussion: The method shown accuracy with a recovery range from 98.89% to 99.86%.

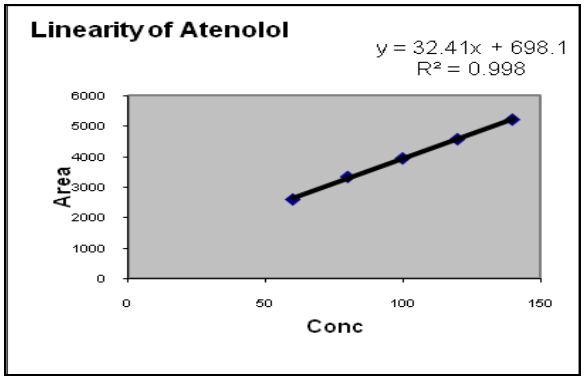
Assay

Atenolol		
Standard Area	1	3898.788
	2	3885.267
	3	3896.237
	4	3856.458
	5	3944.124
	Average	3893.431
Sample area	1	3838.744
	2	3853.108
	3	3819.736
	4	3950.388
	5	3856.382
	Average	3863.672
Standard weight		25
Sample weight		175.6
Average Wt.		175.2
Label claim		25
std.purity		99.8
Assay in mg		**24.70**
%Assay		**98.81**

Discussion: The Formulated sample analysis showed a result of 98.81% of the lable claim.

LOD & LOQ

ppm	Area
60	2601.563
80	3340.869
100	3950.388
120	4580.602
140	5222.845
31.62	1025.49



	ppm	Area
LOD	3.22	104.42
LOQ	9.76	316.41

DISCUSSION

By using the method a LOD of 3.22ppm was found and LOQ of 9.76ppm concentration

CONCLUSION

The new HPLC method developed and validated for determination of Atenolol pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its

solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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