STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATENOLOL, LOSARTAN POTASSIUM AND THEIR DEGRADATION PRODUCTS

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ABSTRACT

A simple, sensitive and specific RP-HPLC method was developed for the simultaneous estimation of Atenolol and Losartan potassium (Losartan K) in tablet dosage form. Separation was achieved with an RP C_{18} (Thermo), 250×4.60 mm 5 micron size column, ambient temperature with a low pressure gradient mode with mobile phase containing methanol and 0.1% ortho-phosphoric acid (65:35v/v). The flow rate was 0.9ml/min and eluent was monitored at 274 nm. The selected chromatographic conditions were found to effectively separate Atenolol and Losartan K 4.05 and7.76 min as a retention time respectively. The proposed method was found to be rectilinear over the range of 10-50 µg/ml for both Atenolol and Losartan K respectively. The drug was subjected to oxidation, acid hydrolysis, alkaline hydrolysis and heat to apply stress condition for degradation. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability.

Keywords: - Losartan, Atenolol, RP- HPLC, stress condition, degradation.

INTRODUCTION

According to USFDA 1987 guideline Stability-indicating Assay methods(SIAMs) were defined as the "Quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured"[1]. The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation products. The discussion also touches upon various critical issues, such as the extent of separation of degradation products, establishment of mass balance, etc., which are important with respect to the development of stability-indicating assays, but are not yet fully resolved. [2]

Losartan potassium (Losartan K) is Losartan K is monopotassium salt of 4-butyl-4- chloro-1-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]- 1H-imidazole-5-methanol[3] and shows angiotensin II receptor Antagonist used as an anti-hypertensive[4]. Atenolol is (RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide [3], which shows beta adrenergic blocker used for hypertension. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Literature survey reveals the availability of several methods for estimation of both Losartan K and Atenolol includes UV, HPLC as alone or in combination with other drugs (4-9). No method has been reported for stability indicating method development for simultaneous estimation of both drugs in binary dosage form. Present work emphasizes on the stability indicating assay method development for simultaneous estimation of Losartan K and Atenolol in their combined dosage form by RP-HPLC.

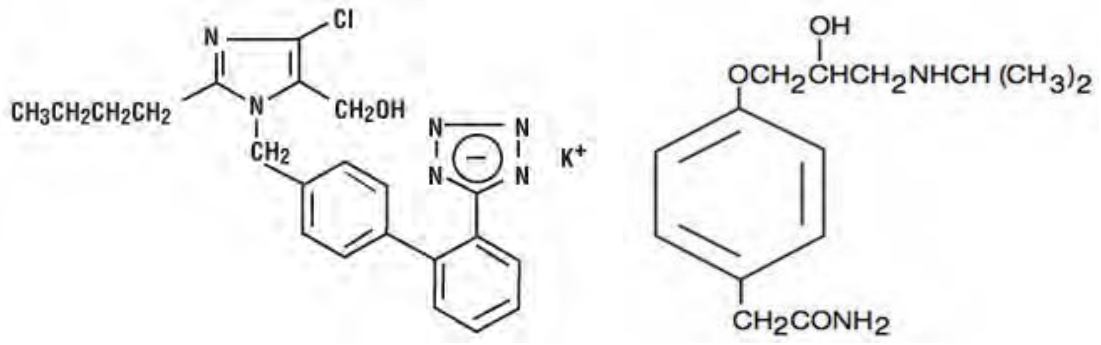

Fig. 1:- Structure of Losartan K (1) and Atenolol (2) [3]

The purpose of this study was to develop a stability-indicating method for the simultaneous determination of Atenolol and Losartan K in bulk drugs and to apply the developed method for the quantitative determination of these drugs from tablets. The HPLC technique was chosen because of its previously mentioned advantages. The proposed method was able to separate the compounds of interest and their degradation products within 10min. Thereafter, this method was validated as per International Conference on Harmonization (ICH) guidelines (10, 11). A literature survey has shown that a stability-indicating HPLC method for the simultaneous determination of Atenolol and Losartan K has not been developed. The previously developed methods have been able to separate both the drugs during a minimum run time, but they were not stability-indicating, i.e., the separation of various degradation products, employing ICH prescribed stress conditions, was not achieved.

In the present research article, we report the development and validation of a stability indicating LC method for the simultaneous determination of atenolol and Losartan K in pharmaceutical dosage form. It separates drug components from degradation products under ICH suggested stress conditions (acid and base hydrolysis, oxidation, photolysis and thermal stress) (10-12)

MATERIALS AND METHODS

Instrument and Chromatographic Condition:-

Chromatographic separation was performed on a Younglin (S.K) Gradient chromatographic system equipped with a Reciprocating pump SP930 D, UV/Visible detector UV 730 D and with 20µL fixed loop and data analyzed by using Autochro-3000software. RP-18, Inertsil ODS column (250 × 4.6 mm×5µ) was used for separation.

Mobile phase consisting of a mixture of methanol: 0.1% O-Phosphoric acid (65:35 % v/v) was delivered at a flow rate of 0.9 ml/min. The mobile phase was filtered through a 0.45µ membrane filter and ultra-sonicated for 10min. Analysis was performed at ambient temperature.

Chemicals and Reagents

Atenolol and Losartan K were obtained as gift samples from Cipla Ltd Mumbai and Micro labs Ltd Banglore respectively. O-phosphoric acid and Methanol were HPLC grade procured from Space Lab. Nashik, Hydrochloric acid, Sodium Hydroxide and Hydrogen Peroxide (AR) grade procured from Loba Chem. Nashik. The pharmaceutical preparations of binary combination of Atenolol and Losartan K that is Losar-Beta tablet (Unichem Laboratories Ltd.). The commercial formulation of Atenolol and Losartan K is available in ratio of 1:1 (50/50 mg)} in tablet.

Preparation of Mobile Phase and Stock Solutions:

Mobile Phase: - the mobile phase was methanol: 0.1% o-phosphoric acid in water (65:35%v/v). This can be sonicated for 10 min. and filtered through 0.22µm membrane filter which is used as a mobile phase.

Stock Solution (1000µg/ml): - Stock solutions were prepared by weighing 10 mg each of Losartan K and Atenolol. The weighed drugs were transferred to two separate 10 ml volumetric flasks and adjust the volume with mobile phase. The stock solutions were sonicated for10min then filtered through Whatman's filter paper.

Working Standard Solutions (100µg/ml): - Take 1 ml solution from stock solution and transfer separately in 10ml volumetric flask and dilute volume up to 10ml with mobile phase.

The HPLC analysis was performed on reversed phase high performance liquid chromatographic system with isocratic elution mode using a mobile phase of methanol: o-phosphoric acid (65:35 v/v) on Spheri-5-RP-18 column (250×4.6 mm, 5 µm particle size) with 0.9 ml/min flow rate at 274 nm using UV detector.

Preparation of Degradation Samples:-

1. Acid hydrolysis: - these samples were prepared by weighing the drugs 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrochloric acid (0.1 N) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath.

2. Alkaline hydrolysis: - these samples were prepared by weighing the samples 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add sodium hydroxide (0.1 N) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath.

3. Oxidation: - these samples were prepared by weighing the samples 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrogen peroxide (3%) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath.

4. Thermal degradation: - these samples were prepared by weighing the samples 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add HPLC grade water and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath.

After degrading the samples, allow it to cool. Take 0.5ml of solution from it and transfer to 10ml volumetric flask. Adjust volume up to 10ml with mobile phase. Each sample filtered through 0.22 µ Millipore filter paper for inject into HPLC.

RESULTS AND DISCUSSION:-

Optimization of Chromatographic Condition: -

The primary target in developing this stability-indicating HPLC method is to achieve the resolution between the Atenolol, Losartan K and its degradation products. Our objective of chromatographic method development was to achieve peak tailing factor < 2, retention time between 3 to 10 min, along with resolution between Atenolol and Losartan K > 2.

Effect of Column Type: -

To achieve the separation of degradation products, we used a stationary phase. This column was found optimum hence; it became the column of choice for this study. The best selectivity was observed on a C18 based stationary phases. The chromatographic separation was achieved using RP C_{18} (Thermo), (250 x 4.6 mm i.d.) column.

Effect of Mobile Phase Mode: -

Changing the composition of mobile phase optimized the chromatographic method. To develop a stability-indicating method it was necessary to maintain proportion of the mobile phase. The effect of mobile phase mode (isocratic and gradient) on the simultaneous separation and determination of Atenolol and Losartan K peaks was studied. Isocratic elution was firstly tried using different Methanol ratios with the 0.1% o-phosphoric acid Low Methanol ratio (60%) resulted in an increase in the retention time of the peaks and this was unsuitable for HPLC, while increasing the Methanol ratio (80%) resulted in decreasing the resolution between the peaks. As a result, the optimized composition of the mobile phase methanol: 0.1% o-phosphoric acid 65:35%v/v was selected.

Effect of Wavelength: -

The wavelength was important for detection of the components for HPLC as a detector. The Atenolol and Losartan K have detection wavelength 275nm and 232nm respectively. The optimum wavelength was selected as 274nm where appropriate detection obtained.

Effect of Flow Rate: -

The flow rate of mobile phase was necessary to maintain because it affects the resolution between both drugs with their degradation products. 09ml/min flow rate was efficient to to resolve all peaks within appropriate time interval. Less flow rate (0.7ml/min) shows maximum resolution but increases the time interval between Atenolol and Losartan K peaks and for 1ml/min shows very less resolution which is inefficient method.In optimized conditions Atenolol and Losartan K and their degradation product were well separated. Typical retention times of Atenolol and Losartan K about 4.05 and 7.7 min respectively. Resolution between Atenolol and Losartan K founds to be 12.1.

Method Validation [3, 12]: -

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

System Suitability:-

System suitability parameters were measured to verify the system performance. System precision was determined on six replicate injections of standard preparations. All important characteristics were measured, including peak resolution, tailing and theoretical plate number.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

The peak purity values were carried out using UV detector. The peak purity was more than 997 for drug substance and drug products at 274 nm, which shows that the peaks of analytes were pure and that formulation excipients and degradation were not interfering with analytes peaks The described method was validated with respective specificity, linearity, system suitability, accuracy, robustness, limit of detection (LOD), limit of quantitation (LOQ)

Linearity and Calibration Curve

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Five point's calibration graphs were constructed covering a concentration range 10–50 µg/ml for both Atenolol and Losartan K. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for Atenolol and Losartan K obtained were $y = 3.816x+1.82$ and $y = 9.152x-8.289$ respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.999 and 0.997 for Atenolol and Losartan K respectively. Relative standard deviation (%RSD) for slope of Atenolol and Losartan K were 1.04 and 0.738 respectively.

Precision (repeatability)

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability expresses the precision under the same operating conditions over a short interval of time.

Accuracy:-

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy of the method was studied by recovery experiments.The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

The recovery was performed at three levels 80%, 100%, and 120% of the label claim of the tablet (50 mg of Atenolol and 50 mg Losartan). Placebo equivalent to one tablet was transferred into a 100-mL volumetric flask and the amounts of Atenolol and Losartan at 80%, 100%, and 120% of the label claim of the tablet were added. The recovery samples were prepared as per the procedure mentioned earlier. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Atenolol and Losartan K ranged from 99.6% to 100.65% and 99.63 to 100.75% respectively. The average recovery and % RSD of three levels (nine determinations) for Atenolol and Losartan were 100.14% (0.62) and 100.01% (0.96) respectively, with %RSD shown in parenthesis.

Limit of Detection and Quantitation (LOD and LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The LODs for Atenolol and Losartan were 1.14 and 0.5 µg/mL, respectively and the LOQs were 3.49 and 1.52 µg/ml respectively for 20-µL injection volume.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness a number of chromatographic parameters (e.g., flow rate, column temperature, injection volume, detection wavelength or mobile phase composition) are varied within a realistic range and the quantitative influence of the variables is determined.

To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Atenolol and Losartan K were evaluated. The flow rate of the mobile phase was 0.9 ml/min. To study the effect of flow rate on resolution of Atenolol and Losartan K, it was changed to 0.1 units from 0.8ml/min and 1ml/min. While other mobile phase components were held constant. The effect of mobile phase composition on resolution of Atenolol and Losartan K was studied with methanol: 0.1% o- phosphoric acid at 64:36 (v/v) and 66:34 (v/v). The effect of wavelength on resolution of Atenolol and Losartan K was studied with applying wavelengths 273nm and 275nm.

Table1: -Optimized Chromatographic conditions

Parameter	**Optimized condition**
Instrument	Younglin (S.K) Gradient System UV Detector
Drugs	Atenolol and losartan potassium
Column	4.6 x 250 mm
Mobile phase	Methanol : 0.1 % o- phosphoric acid(65:35)
Flow rate	0.9ml/min
Detection	274nm
Injection volume	20µl
Temperature	Ambient
Retention time	4.05 and 7.76 for Atenolol and Losartan K respectively.

Table 2:- Regression Characteristics and System Suitability Parameters of Proposed RP-HPLC method

Parameters	**Atenolol**	**Losartan potassium**
Retention time	4.05	7.76
Tailing factor	1.4	1.4
Resolution	12.1	
Theoretical plates	4608.6	6842.9
Linearity (µg/ml)	5-50	5-50
LOD (µg/ml)	1.14	0.5
LOQ (µg/ml)	3.49	1.52
Regression Equation	y = 3.816x+1.82	y = 9.152x-8.289
Slope(m)	3.816	9.152
Intercept(c)	1.82	-8.289
Correlation coefficient (r^2)	0.999	0.998
% RSD	1.04	0.738

Table 3: Robustness study

Parameters	**Resolution**
Flow rate (ml/min)	
0.8	10.723
0.9	12.46
1	11.68
Wavelength (nm)	
275	10.957
274	12.46
273	10.516
Mobile phase composition (v/v)	
64:36	10.02
65:35	12.46
66:34	11.57

Table 4:- Results of the Recovery Tests for theAtenolol and Losartan K

Level of addition (%)	Drugs	Tab. Conc. (n=3) µg/ml	Amount added (n=3) µg/ml	Average Amount recovered	% recovery
80	Atenolol	20	16	16.05	100.33
	Losartan K	20	16	15.94	99.63
100	Atenolol	20	20	20.02	100.08
	Losartan K	20	20	20.13	100.65
120	Atenolol	20	24	24.006	100.02
	Losartan K	20	24	23.94	99.75

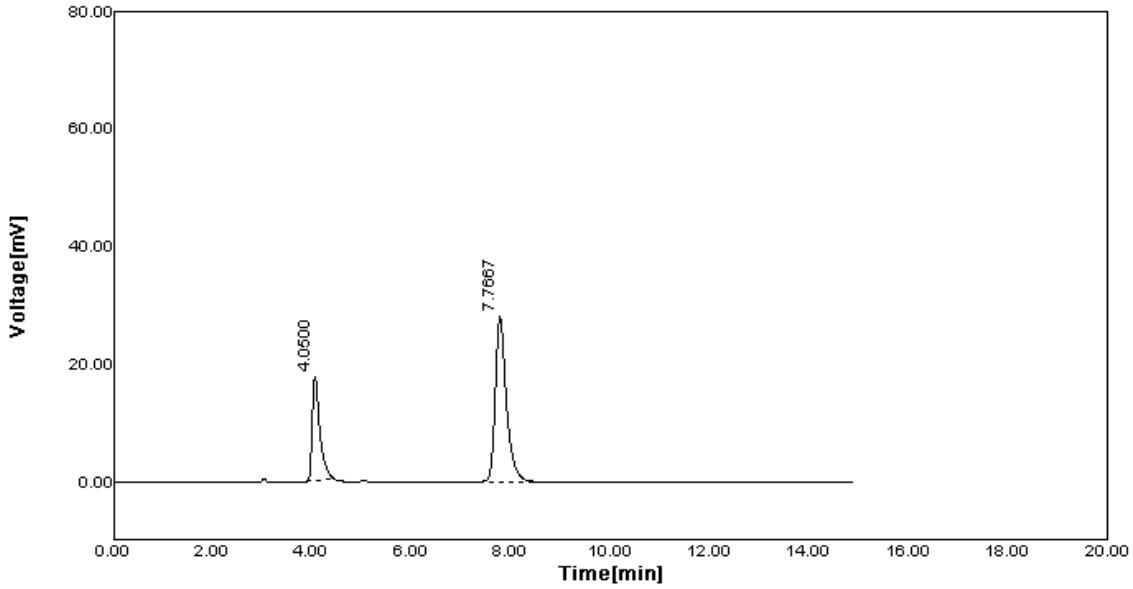

Fig. 1: - Chromatogram Of Separation Between Atenolol And Losartan K.

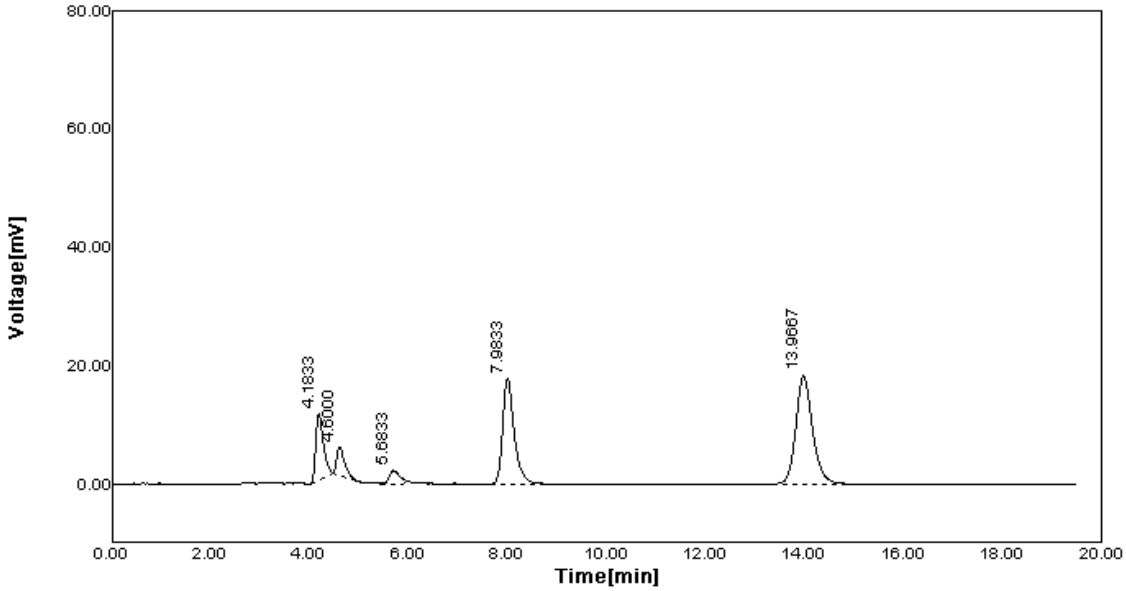

Fig. 2: - Chromatogram of Acid Hydrolysis Of Atenolol And Losartan K.

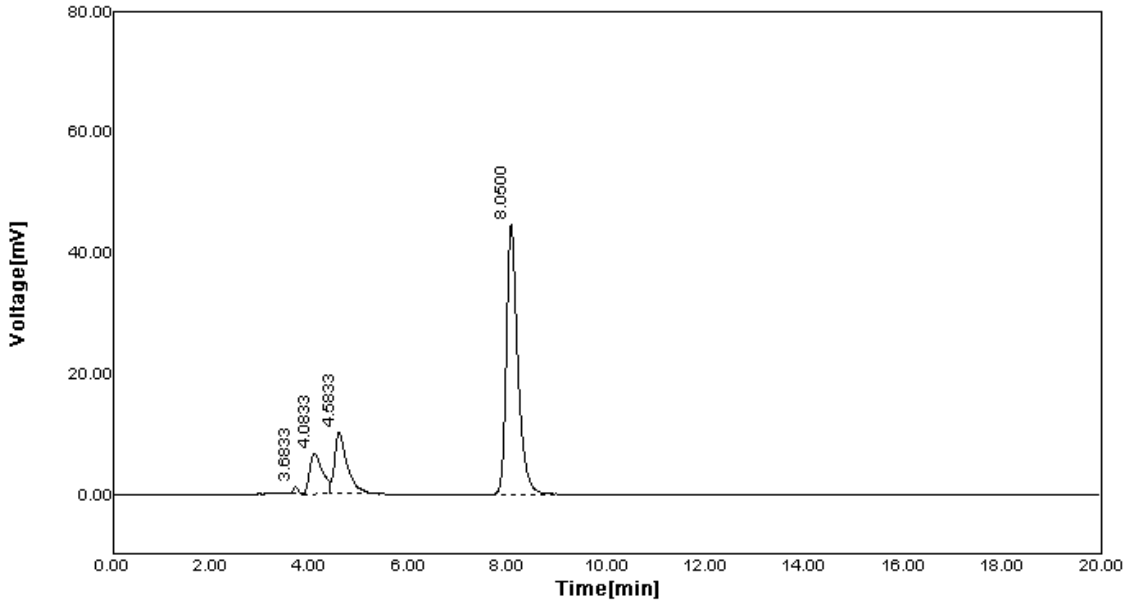

Fig. 3: - Chromatogram of Alkaline Hydrolysis Of Atenolol And Losartan K.

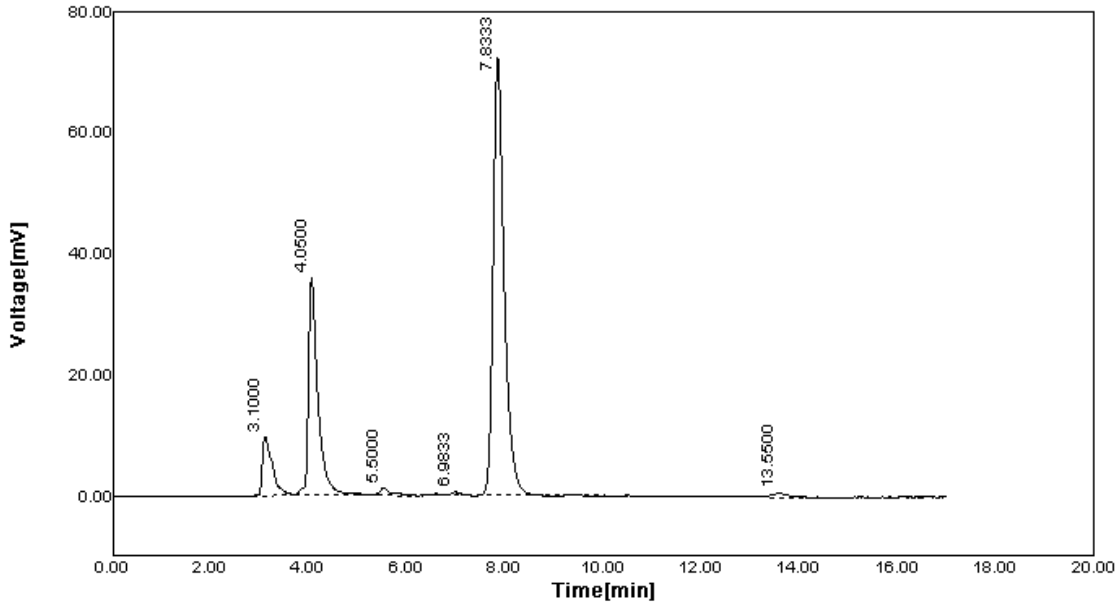

Fig. 4: - Chromatogram of Oxidative Degradation Of Atenolol And Losartan K.

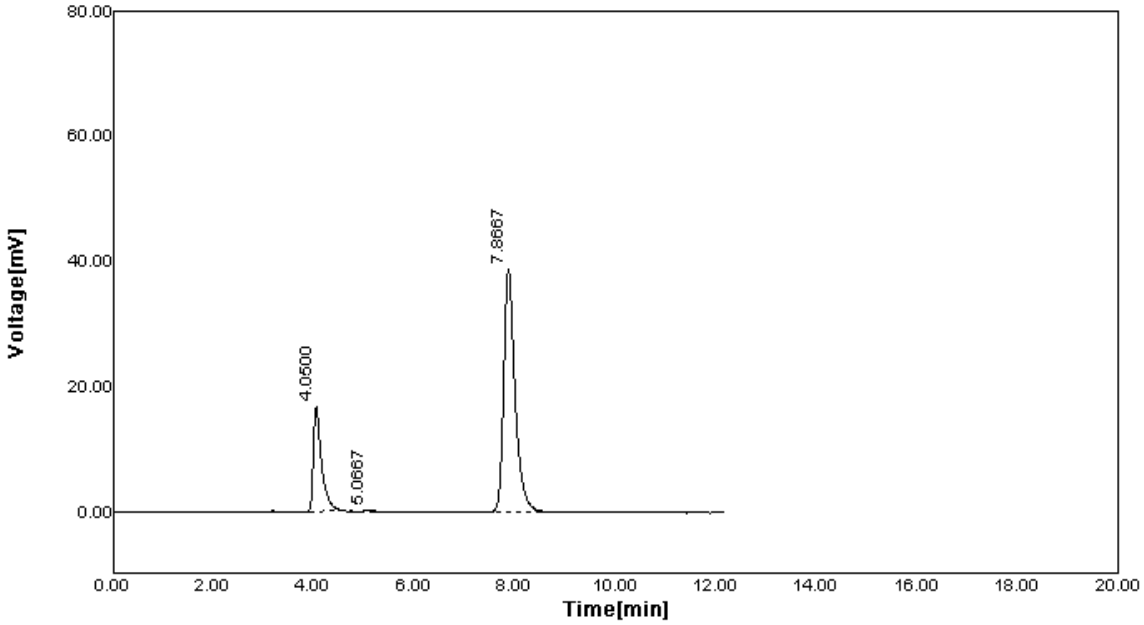

Fig. 4: - Chromatogram of Thermal Degradation Of Atenolol And Losartan K.

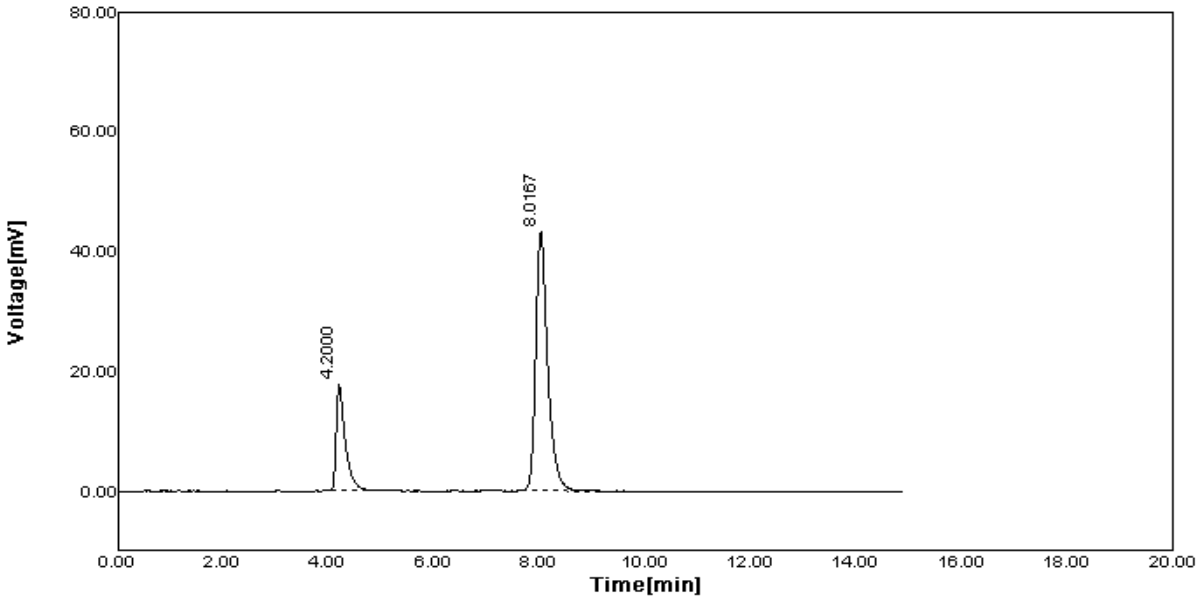

Fig. 4: - Chromatogram of Neutral Degradation Of Atenolol And Losartan K.

CONCLUSION

A new analytical method was developed to be routinely applied to simultaneous determination of Atenolol and Losartan K in pharmaceutical dosage form. In this study, the stability of Atenolol and Losartan K in present dosage forms was established through employment of ICH recommended stress conditions. The developed procedure was evaluated for specificity, linearity, accuracy, precision and robustness to ascertain the stability of the analytical method. The method was proved to be specific, linear, precise, accurate, robust and stability-indicating. Hence, the method is recommended for routine quality control analysis and stability sample analysis.

ACKNOWLEDGEMENT:-

The authors are grateful to the Micro labs Bangalore and Cipla Pharmaceuticals Mumbai for gift samples (Atenolol and Losartan K).The authors also thankful to Space lab supplying the HPLC grade chemicals. Authors also thankful to Amrutvahini college of pharmacy college, Sangamner for permitting practical lab.

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