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Analytical Method Development and Validation of Losartan Potassium and Atenolol in combined dosage form by RP-HPLC

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Abstract: A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Losartan Potassium and Atenolol from combined dosage form by reverse phase C_{18} column (Phenomenex C_{18}, 5µ, 250mm x 4.6mm). The sample was analysed using Triethylamine: Acetonitrile: Methanol in the ratio of 50:30:20(pH adjusted to 4.0 with phosphric acid) as a mobile phase at a flow rate of 1.2ml/min and detection at 235nm. The retention time for Losartan Potassium and Atenolol was found to be 3.767 min and 2.210 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form.

Keywords: Losartan Potassium , Atenolol, RP-HPLC.

Introduction

Losartan Potassium[1] is a Angiotensin II receptor Antagonist used as an anti-hypertensive. Atenolol[2] is a 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 Potassium[3-8] and Atenolol[9-11] includes UV, HPLC as alone or in combination with other drugs. No method has been reported for the estimation of Losartan Potassium and Atenolol in combined dosage form. Present work emphasizes on the quantitative estimation of Losartan Potassium and Atenolol in their combined dosage form by RP-HPLC.

Experimental

A High Performance Liquid Chromatograph system, The purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter (Phenomenex C_{18}, 5µ, 250mm x 4.6mm). Optimized chromatographic conditions are listed in Table 1.

Materials and Chemicals:

Pure samples of Losartan Potassium and Atenolol were obtained from Rakshi drugs Pvt.Ltd. and Shanpur Pharma chem. respectively for the estimation of Losartan Potassiumn and Atenolol in commercial formulations. HPLC grade phosphric acid, Acetonitrile and Methanol were procured from Qualigens fine chemicals. High pure water prepared by using Millipore Milli Q plus purification system.

Preparation of Standard stock solution:

Solution (A): Weighed accurately 100mg of Losartan Potassium working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well.

Solution (B): Weighed accurately 100mg of Atenolol working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well.

Mixtured standard solution: Diluted 5ml of Solution (A) and 5ml Solution (B) to 50ml with mobile phase.

Preparation of Sample solution:

Weighed and finely powdered not less than 20 tablets. Transferred an accurately weighed portion of the powder equivalent to about 100mg to 100ml volumetric flask, added 70ml of mobile phase. Sonicated for 15min and cooled to room temperature. Diluted to 100ml with mobile phase. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate.

Injected separately 20µl of the standard preparation in to the equilibrated HPLC system in 5 replicate and measured the response of the major peak due to Losartan Potassium and Atenolol. Then injected separately 20µl of the sample preparation in to duplicate and measured the response of the major peak due to Losartan Potassium and Atenolol.. And calculated the content of Losartan Potassium and Atenolol..

Validation of the Method[12]

The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Losartan Potassium, Atenolol and measured the absorbance at 235nm. Calibration curves where constructed by plotting the area against the concentration. Losartan Potassium shows the linearity in the concentration range from 80-120 µg/ml with correlation coefficient of 0.9999 and Atenolol shows the linearity in the concentration range from 80-120 µg/ml with correlation coefficient of 0.9998.

Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to preanalysed tablet powder, at three level and the percentage recoveries were calculated. Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions.

Results and Discussion

1. Estimation

A RP-HPLC method was developed for the simultaneous estimation of Losartan Potassium and Atenolol in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solutions were prepared and chromatograms were recorded.

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard deviations in formulation were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

2. Validation of the method

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in Table 3. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and percentage relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature.

The standard drug solutions in varying concentrations ranging from 80 to 120 % of the targeted level of the assay concentration were examined by the assay procedure. Losartan Potassium and Atenolol were found to be linear in the range of 80 to 120 µg/ml and 80-120 µg/ml respectively.

The slope, intercept and correlation coefficient values were also calculated. The correlation co-efficient of Losartan Potassium and Atenolol were found to be 0.9999 and 0.9998 respectively. The calibration curves were plotted as peak area Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration

of the analytes. The range demonstrates that the method is linear outside the limits of expected use.

The additional peaks were observed in the chromatogram of the formulation, which may be due to excipients present in the formulation. These peaks do not interfere with the standard peaks, which clearly confirm the assay method was found to be highly specific.

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the develop methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Losartan Potassium and Atenolol were found to be 4.40 µg /ml and 4.07µg /ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Losartan Potassium and Atenolol were found to be 13..8µg /ml and 12.33µg /ml respectively.

The system suitability studies were performed for the standard solutions and were presented in Table 2. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analytical process.
- Suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 15 times.

Hence, the chromatographic method developed for Losartan Potassium and Atenolol were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

Table 1: Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	WATERS ALLIANCE 2695
Column	Phenomenex C_{18},5µ,250mm x 4.6mm
Mobile phase*	Buffer : Acetonitrile : Methanol (50 :30:20 V/V)
Flow rate	1.2 ml/min
Detection	235nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated.

Table 2: System Suitability Parameters

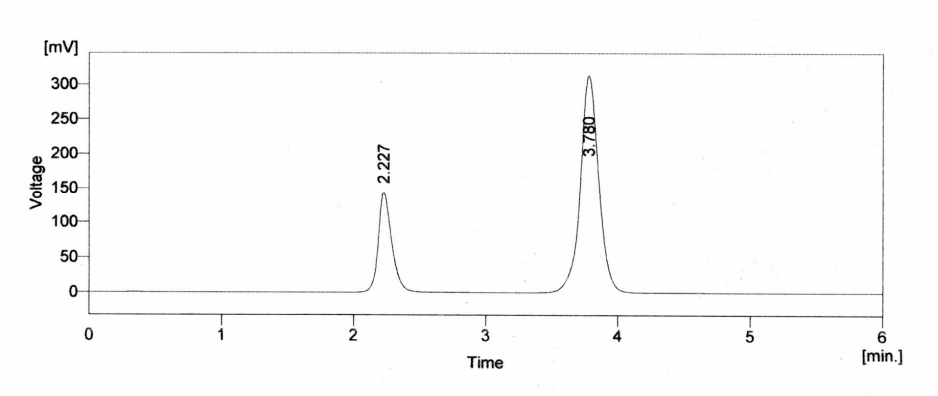
Parameter	Losartan Potassium	Atenolol
Theoretical plates	3826	2534
Resolution	7.427	
Tailing factor	1.068	1.538
LOD (µg/ml)	4.40 µg /ml	4.07µg /ml
LOQ (µg/ml)	13..8µg /ml	12.33µg /ml

Table 3: Analysis of Formulation and Recovery studies

Drug	Label claim (mg/ml)	*Estimation		**Recovery
		mg/tablet	Amount added(µg/ml)	% recovery
Losartan Potassium	50mg	49.6mg	3.90	98.48%
			4.947	99.90%
			5.90	100.16%
Atenolol	50mg	49.1mg	9.858	99.37%
			12.27	98.95%
			14.70	98.80%

*mean (%RSD) of five observations, **mean (%RSD) of three determinations.

Figure 1: Typical chromatogram of Losartan Potassium and atenolol.



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