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SIMULTANEOUS DETERMINATION AND METHOD DEVELOPMENT FOR ASSAY OF LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE DRUGS IN SOLID DOSAGE FORM BY RP-HPLC

Khan M. Rizwan[1], Shaikh Anis[1], Thaker A.K.[2]

[1]Institute of Pharmacy, Vikram University, Ujjain-456001.
[2] School of Pharmacy and Technology Management, SVKM's NMIMS University, Vile-Parle (w), Mumbai-400056.

ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed for the simultaneous determination of Losartan Potassium (LOS) and Hydrochlorothiazide (HCTZ) from combined dosage form by reverse phase C18 column (Zorbax CN (250mm x 4.6mm) 5µ). The sample was analysed using Triethylamine: Acetonitrile: Methanol in the ratio of 33:27:40(pH adjusted to 7.0 with phosphric acid) as a mobile phase at a flow rate of 1.0ml/min and detection at 270nm. The retention time for Losartan potassium (LOS) and Hydrochlorothiazide (HCTZ) was found to be 11.869 min and 7.893 min respectively. The stability assay was performed for this combination and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the 70%-130% concentration ranges for both the drugs of LOS and HCTZ 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, Hydrochlorothiazide, RP-HPLC.

INTRODUCTION

Losartan Potassium [1] is a Angiotensin II receptor Antagonist used as an anti-hypertensive. Hydrochlorothiazide [2] is a Loop Diuretics used as an anti hypertensive by reducing symtomatic oedema. 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 Hydrochlorothiazide [9-11] includes UV, HPLC as alone or in combination with other drugs. Method has been reported for the estimation of Losartan Potassium and Hydrochlorothiazide in combined dosage form. Present work emphasizes on the stability testing of Losartan Potassium and Hydrochlorothiazide in their combined dosage form by RP-HPLC. Literature survey reveals that there is method developed for the combination Losartan Potassium and Hydrochlorothiazide [8].

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 reverse phase C18 column (Zorbax CN (250mm x 4.6mm) 5µ). Optimized chromatographic conditions are listed in Table 1.

Corresponding Author:- **Khan M. Rizwan** Email:- khan_rizwanr@yahoo.co.in

Materials and Chemicals

Pure samples of Losartan Potassium and Hydrochlorothiazide were obtained from Unichem Pvt.Ltd. for the estimation of Losartan Potassiumn and Hydrochlorothiazide in commercial formulations. HPLC grade phosphric acid, Acetonitrile and Methanol were procured from institute and of Rankem ltd. High pure water prepared by using Millipore Milli Q plus purification system.

Preparation of Standard Stock Solution

Solution A: Weigh accurately about 50 mg of Losartan Potassium working standard in a 100 ml volumetric flask. Dissolve and dilute up to mark with diluent. (500ppm)

Solution B: Weigh accurately about 25 mg of Hydrochlorothiazide working standard in a 100 ml volumetric flask. Dissolve and dilute up to mark with diluent. (250ppm)

Mixtured Standard Preparation

Pipette out 10 ml of solution A and 5 ml of solution B into a 100 ml volumetric flask. Make the volume up to mark with diluent. (50ppm of Losartan potassium and 12.5ppm of Hydrochlorothiazide)

Preparation of test sample

Sample Stock Preparation

Weigh and transfer 5 tablets in a 250 ml volumetric flask. Add about 150ml of diluent sonicate till the tablet get disperse completely shake and sonicate extra 10min after tablet get disperse with vigorous shaking cool and make up the volume with diluent..

Sample Preparation

Take 5ml of the sample stock solution in 200ml flask and make up the volume with diluent. Filter the solution through 0.45 nylon membrane filter paper.

(50ppm of Losartan potassium and 12.5ppm of Hydrochlorothiazide)

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, Hydrochlorothiazide and measured the absorbance at 270nm. Calibration curves where constructed by plotting the area against the concentration. Losartan Potassium shows the linearity in the concentration range from 35-65ppm with correlation coefficient of 0.9999 and Hydrochlorothiazide shows the linearity in the concentration range from 8.75-16.75ppm 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 reanalyzed 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.

Table 1. Optimized Chromatographic conditions

Parameter	**Optimized condition**
Instrument	Waters HPLC/Empower software/PDA detector
Column	Zorbax CN (250mm x 4.6mm) 5µ
Mobile phase*	Triethylamine: Acetonitrile: Methanol in the ratio of 33:27:40(pH adjusted to 7.0 with phosphric acid)
Flow rate	1.0ml/min
Detection	**270nm**
Injection volume	20µl
Temperature	Ambient

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

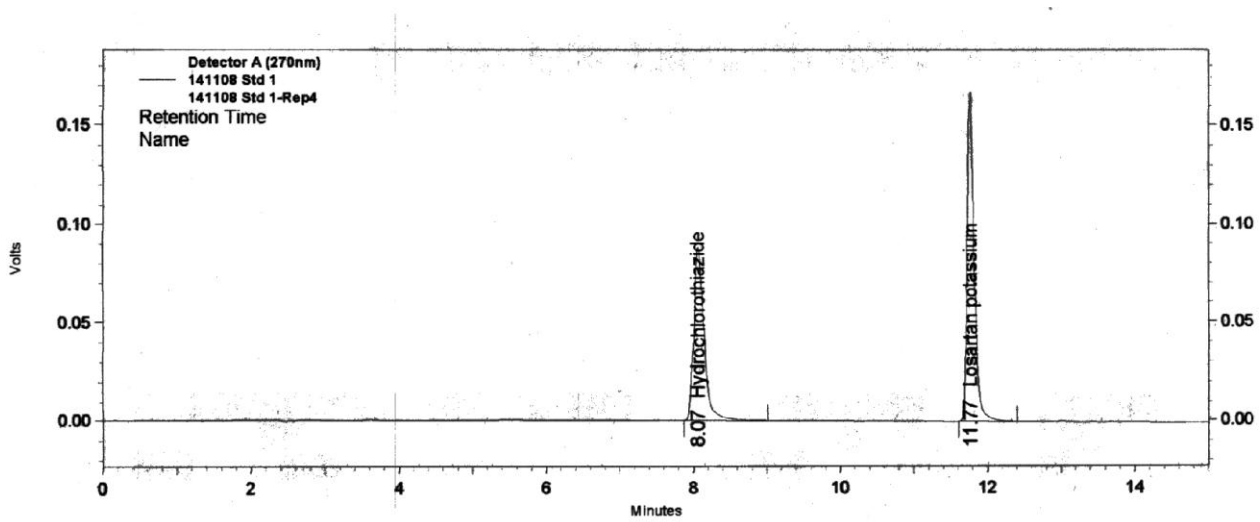
Table 2. Analysis of Formulation and Recovery studies

Assay No.	**Mean Peak Area for Losartan**50ppm	**Losartan Potassium Assay (%)**	**Mean Peak Area for HCTZ** 12.5ppm	**HCTZ Assay (%)**
1	847410	102.2	966274	99.8
2	852145	102.8	973023	100.5
3	840808	101.4	960635	99.2
4	860665	103.8	984912	101.7
5	847918	102.3	969477	100.1
6	847101	102.2	968552	100.0
Mean	849341.02	102.5	970478.8	100.2
% RSD	0.78	0.78	0.84	0.84

Table 3. System Suitability Parameters

System Suitability test				
Analytes	RT(N=5)	Tailing Factor(N=5) Limit(NMT 2.0)	Theoretical Plates (N=5) Limit (NLT 2000)	%RSD (N=5) Limit (NMT (2.0)
Losartan potassium	11.85	1.03	19440	0.44
HCTZ	7.88	1.02	57704	0.47

Figure 1. Typical chromatogram of Losartan Potassium and Hydrochlorothiazide



RESULTS AND DISCUSSION

1. Estimation

A RP-HPLC method was developed for the simultaneous estimation of Losartan Potassium and Hydrochlorothiazide 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 70%-130% 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 2. 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 70% to 130 % of the targeted level of the assay concentration were examined by the assay procedure. Losartan Potassium and Hydrochlorothiazide were found to be linear in the range of 35 to 65ppm and 8.75-16.25ppm respectively.

The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of Losartan Potassium and Hydrochlorothiazide 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 system suitability studies were performed for the standard solutions and were presented in Table 3. 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.

Ø The standard and sample preparation requires less time.
Ø No tedious extraction procedure was involved in the analytical process.
Ø 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 Hydrochlorothiazide 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.

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