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Research Article

Simultaneous Determination of Losartan Potassium in Pharmaceutical Products by Reversed Phase High Performance Liquid Chromatography

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ABSTRACT

Objective: Method validation is an important aspect for the determination of pharmaceutical products. A well validated method play an important role in the control of quality of the products. So this purpose the proposed study have been done regarding develop a validated method for losartan potassium. Methods: A reversed phase high performance liquid chromatographic method used on isocratic mode. Results: The chromatographic separation of losartan potassium was obtained using a C_{18} column by isocratic elusion at the 25^0C column temperature. The green solvent (methanol and water, 30:70 v/v) was used as a mobile phase. The analysis was performed at the flow rate 1.0 ml/min. A well defined peak was detected at 273 nm. The retention time of acive engredients losartan potassium was obtained in 7 min. The limit of detection and limit of quantification was calculated 0.03 and 0.09 µg/ml, respectively. A Good results was obtained with respect to linearity R^2=0.998. The mean recoveries in inter-day and intra-day were calculated 98.85 % and 99.4 % with CV value 1.38 and 2.24 respectively. Conclusion: The method was validated according to ICH guidelines. This method is very efficient for the analysis of losartan potassium at 25 ^{0}C.

Keywords: RP-HPLC, Losartan potassium, recoveries.

INTRODUCTION

Losartan potassium generally used in the treatment of hypertention, heart failureand heart attacks and it is also prevent to the complications of diabetes. It is angiotensin converting enzyme inhibitor. Chemically it is 2-butyl-4-chloro-1-{[2-(1H-tetrazol-5-yl]methyl}-1H-Imidazol-5-yl)methanol and the empirical formula is $C_{22}H_{22}ClKN_6O$[1,2].

The literature survey has been shown various analytical methods for the estimation of losartan potassium active ingredients using high performance liquid chromatography methods[3-5]. The proposed study has been done according to ICH guidelines[6-8].

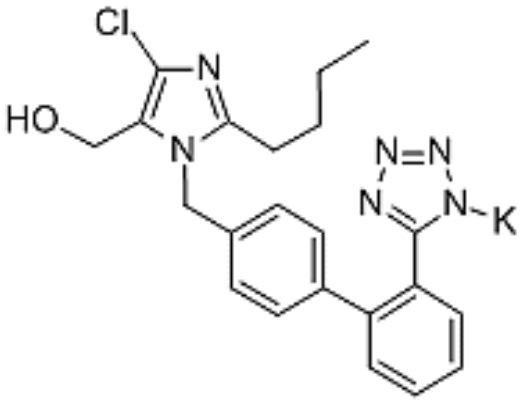

Fig.1: Chemical structure Losartan Potassium

MATERIALS AND METHODS

Preparation of mobile phase

A methanol / water mobile phase was prepared in the ratio of methanol: water (30:70, v/v). The mobile phase were filtered through a 0.45µm nylon membrane and degassed by sonication.

Preparation of Losartan potassium stock solution

A 100 µg/ml stock solution was prepared, 10 mg losartan potassium was mixed with methanol and make up it to 100 ml. The stock solution was filtered with 0.45 µm nylon filter membrane and degassed by sonication.

Preparation of sample solution

The 10 tablets of Losartan potassium were weight and crushed by mortar and pestle. The crushed tablets were mixed well, and then an equivalent amount of 10 mg was transferred in to a small conical flask and extracted with methanol ratio. The extract was filtered into a 100 ml

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Table No. 1. Results for system suitability

S. NO.	Parameter	Mean value	CV in Percentage (%)
1.	Retention Time	7.1	2.032
2.	Tailing factor	0.86	0.310
3.	Resolution factor	1.561	0.72
4.	Theoretical factor	658.004	3.788

CV = coefficient variance

Table No. 2. Results for linearity

S.NO.	Parameter	RP-HPLC
1.	Correlation factor	10-50µg/ml
2.	Liner equation	13852X-43291
3.	Regression coefficient (R^2)	0.997

volumetric flask and the volume make up to 100 ml. Achieved aliquots was covered the working concentration range 100 µg/ml.

Preparation of calibration curve

A calibration curve was constructed to evaluation the linearity. The calibration curve was plotted, using average peak area ratio and different drug concentrations (µg/ml). A total volume of 10 ml was maintained with mobile phase methanol and water. These different serial dilutions were filtered through a 0.45µm nylon membrane and sonicate. The each solution of 20 µl was injected into the column in thrice. The calibration curve was obtained by plotting peak area versus concentration.

Method validation

The describe method was validated according to ICH guidelines with respect to following parameters.

Linearity

As per ICH guidelines the linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Linearty can be calculated by using following equation as per ICH guidelines.

$y = mx + b$

Where 'm' and 'b' are the constants, the constants 'm' determines the slop or gradient of that line and the constant 'b' determines the point at which the line crosses the y-axis, otherwise known as the y- intercept.

Specificity

It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Specificity can be calculated by using following equation as per ICH guidelines.

$$Specificity = \frac{Number\ of\ true\ negatives}{Number\ of\ true\ negatives + number\ of\ false\ positives}$$

Specificity was determined with excipients of formulated tablets. An equivalent weight was taken and solution prepared similarly to the sample solution. The prepared solution was determined as per the describe method.

Table No. 3 Limit of detection (LOD) and limit of quantification (LOQ)

S.NO.	Parameters	RP-HPLC
1.	Limit of Detection (LOD)	0.03 µg/ml
2.	Limit of Quantification (LOQ)	0.09 µg/ml

Accuracy

The accuracy of the proposed methods was checked by recovery studies. The accuracy measured by addition of standard drug solution to reanalyzed sample solution at three different concentration levels within the range of linearity.

Accuracy can be calculated by using following equation as per ICH guidelines.

$$\%Recovery = \frac{Peak\ Area\ of\ the\ Drug\ in\ standard}{Peak\ Area\ of\ the\ Drug\ in\ sample\ Mixture} \times 100$$

System suitability test

The reproducibility of sample was checked of the sample to measurement of peak area and was carried out using three replicates of same concentrations of standard and sample, respectively.

Limit of detection (LOD) & limit of quantification(LOQ)

Detection of 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 limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times \frac{Standard\ deviation\ of\ the\ Peak\ Area\ of\ the\ drug}{Slope\ of\ the\ Corresponding\ Calibration\ Curve}$$

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOQ = 10 \times \frac{Standard\ deviation\ of\ the\ Peak\ Area\ of\ the\ drug}{Slope\ of\ the\ Corresponding\ Calibration\ Curve}$$

To determine the limit of detection (LOD) and limit of quantification (LOQ) prepared three replications of low concentrations serial dilution of mixed standard of losartan potassium from the standard stock solution.

RESULTS AND DISCUSSION

Selection of mobile phase and Chromatographic conditions

It is a basic need of high performance liquid chromatography (HPLC). The mobile phase have been selected to check the mobile phase at various composition with the solvent of HPLC grade water and methanol 70:30, 60:40, 50:50, 40:60 and 30:70 (v/v). The mobile phase methanol and water in the ratio of 30:70 (v/v) are selected, thus at this mobile phase was obtained a suitable retention time and peak area of the active ingredients. The separation achieved on reversed phase C_{18}- column with the wave length 273 nm.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo, there is no interference was observed for any of the components

Table No. 4. Results of inter day recovery experiment

SR.NO.	Drug added in µg	Recovery in µg	Recovery in %	Mean %	CV %
1.	10	9.88	98.8		
2.	20	20.05	100.25	98.85	1.38
3.	30	29.25	97.5		

Table No. 5. Results of intraday recovery experiment

S. NO.	Drug added in µg	Recovery in µg	Recovery in %	Mean %	CV %
1.	10	9.9	99		
2.	20	20.5	102.5	99.94	2.24
3.	30	29.5	98.33		

CV = coefficient variance

like excipients.

System stability test

System suitability test was performed to stabilization the chromatographic conditions. The test was performed by injecting the standard mixture in thrice replications. The various parameters Retention time (R_t), Tailing Factor (T_f), Resolution Factor (R_f) and Theoreitical plates (T_p) were computed. All parameters were statistically calculated. The CV% of the retention time (R_t), tailing factor (T_f), resolution factor (R_f) and theoretical plates (T_p) were reported 2.032, 0.310, 0.72, and 3.788 which were shown in the following table[9-10].

The calculated CV % value of all parameters are less than 10 (<10), thus all CV% values are significant[11].

Linearity

The detector response for the proposed method determined to be linear over the range. The five concentration levels 10, 20, 30, 40 and 50 µg/ml were prepared and injected. The calibration curve was plotted between drug concentration and average peak area for. The linearity of the method was calculated by linear regression analysis.

The correlation range was determined 10-50 µg/ml. The regression coefficient was calculated 0.997. Hence the above calculated data are showing that the detector response is linear.

Limits of Detection (LOD) and Limit of Quantification (LOQ)

The limlt of detection (LOD) and limit of quantification (LOQ) were determined by calculating the signal to noise ratio for losartan potassium. The limit of detection (LOD) and limit of quantification value was found 0.03µg/ ml and 0.09 µg/ ml.

Accuracy

The accuracy of the method was determined by recovery method at the three concentration levels (10, 20 and 30 µg). The recovery study has been done on the basis of inter day and intraday. The results of inter day recoveries was calculated 98.85 % with CV value 1.38 % and in intraday calculated 99.94 % with CV value 2.24%.

The CV % in both experiments interday and intraday was estimated less than 10 which indicated that the experiment is significant. Thus the recovery results were shown good co-relation with adding losartan potassium drug content. Thus, experimental method was success for the quantitative determination of Losartan potassium[12].

CONCLUSION

The proposed study has been done in isocratic mode with reversed phase-high performance liquid chromatography method. The developed method was linear as shown the calculated value (R^2) 0.997. The limit of detection and limit of quantification was calculated 0.03 and 0.09 µg/ml. The inter-day and intra-day average percentage recovery at three concentration levels were estimated 98.85 % and 99.94 % with the coefficient variation percentage 1.38 (<10) and 2.24 (<10). Therefore, this method is very efficient for the estimation of drug content from pharmaceutical products.

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