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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LOSARTAN POTASSIUM IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Losartan Potassium in tablet dosage form. Chromatography was carried out on a pre-packed Spherisorh C18, 250 X 4.6mm, 5µ using filtered and degassed mixture of Acetonitrile: Phosphate buffer (350:650) as mobile phase at a flow rate of 1.5ml/min and effluent was monitored at 254nm. The method was validated in terms of linearity, precision, accuracy, and specificity, robustness and solution stability. The method does require only 10 minutes as run time for analysis which prove the adoptability of the method for the routine quality control analysis of the drug.

Key words: Losartan Potassium, RP-HPLC, Method development, Validation, Chromatogram.

INTRODUCTION

Losartan Potassium is monopotassium salt of 4-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol and Losartan is a selective, competitive Angiotensin II receptor type 1 (AT_1) receptor antagonist, reducing the end organ responses to angiotensin II. Losartan administration results in a decrease in total peripheral resistance (afterload) and cardiac venous return (preload) All of the physiological effects of angiotensin II, including stimulation of release of aldosterone, are antagonized in the presence of losartan. Reduction in blood pressure occurs independently of the status of the renin-angiotensin system. As a result of losartan dosing, plasma renin activity increases due to removal of the angiotensin II feedback. The literature survey [1-2] reveals that there is some HPLC methods have been reported. In this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the determination of Losartan potassium in tablet dosage form.

EXPERIMENTAL WORK

Working standard of Losartan Potassium, HPLC grade acetonitrile, Merck grade Tetra ammonium dihydrogen phosphate, and Milli-Q water were obtained from Market. The separation was carried out on isocratic HPLC system Waters 2695 with UV detector with pre-packed Spherisorh C18, 250 X 4.6mm, 5µ using filtered and degassed mixture of Acetonitrile: Phosphate buffer (350:650) as mobile phase.

Preparation of ammonium dihydrogen phosphate buffer pH 3.0

Weigh about 8.62 g ammonium dihydrogen phosphate to a 1000 ml volumetric flask, dissolve and dilute to volume with water and mix. Adjust to pH 3.0 ± 0.1 with phosphoric acid.

Preparation of mobile phase

Prepare a mixture of buffer and acetonitrile (65:35). Filter through 0.45µ membrane filter and degas.

Preparation of standard solution

Weight accurately about 50 mg of Losartan Potassium WS in 50 ml volumetric flask. Add about 40 ml mobile phase, sonicate to dissolve and dilute to volume with mobile phase and mix. Further dilute 10.0 ml of this solution to 50 ml with mobile phase and mix.

Chromatographic conditions

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Flow rate 1.5ml/min; detection wavelength 254nm; injection volume 20µl; column used Spherisorh C18, 250 X 4.6mm, 5µ; column temperature: 25°C; mobile phase: Acetonitrile: Buffer (350:650).

Method development [3-6]

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation

Weight accurately about 306.4 mg Losartan Potassium and transfer it in to a 100 ml volumetric flask. Add about 80 ml water, sonicate to dissolve and dilute to volume with water and mix. Filter through Whatman filter paper no. 1. Discard first few ml of the filtrate. Further dilute 10.0 ml of the filtrate to 50 ml with Mobile phase and mix. Filtered the solution through 0.45micron membrane filter and collected the filtrate after discarding the first few ml of the filtrate.

Procedure

20µl of the standard preparation and assay preparation were separately injected and chromatographed. The results obtained for Losartan potassium were shown in Table 1.

METHOD VALIDATION [7-9]

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.,

Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one components (e.g. diode array, mass spectrometry).

Preparation of Placebo

Placebo is prepared by mixing all excipients without active ingredient.

Determination

Weighed accurately 206.4 mg of placebo and transferred it into a 100ml volumetric flask, dissolved with sufficient diluent and made up to the volume with the same. Filtered the above solution by using Millipore filter paper, add 50ml of mobile phase and sonicate to dissolve with Mobile phase and make up to the volume. After the filtrate solution nylon Millipore filter paper. The 20µl of this solution was injected and chromatogram was recorded.

Linearity

To get a concentration of 60%, 80%, 100%, 120% & 140%, of drug pipetted out. 6ml, 8ml, 10ml, 12ml and 14 ml of mixed standard stock solution into separate 25ml volumetric flasks labeled as linearity 60%, linearity 80%, linearity 100%, linearity 120%, & linearity 140%. The volume was made up with diluent. From these different solutions 20µl was injected individually and the chromatograms were recorded. There exists a liner relationship in the two graphs for the two concentration ranges, which are prepared.

Linearity was demonstrated by analysing five different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Losartan Potassium which were found to be linear in the range of 120mcg/ml-280mcg/ml. Coefficient of correlation was 0.999 (Fig-2).

Recovery

Different known concentrations of standard at 3 different levels were added to the blank matrix i.e placebo, the recovery was found to be more than 99.00%. The results obtained for Losartan Potassium were shown in Table 4.

Precision

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.

System Precision

The system precision was evaluated by measuring the peak response of Losartan Potassium of the working standard solution prepared as per the proposed method and chromatograms were recorded and shown in fig. Standard deviation and relative standard deviation was calculated.

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

Determination

Weight accurately about 50mg of Losartan potassium in-house reference standard and transferred it into 100ml volumetric flaks. Add about 40 ml mobile phase, sonicate to dissolve and dilute to volume with mobile phase and mix. Further dilute 10.0 ml of this solution to 50 ml with mobile phase and mix.

Ruggedness

The Ruggedness of an analytical method is determined by the analysis of aliquots from homogeneous lots in different laboratories, by different analysts, using operational and environmental condition that may differ but are still within the specified parameters of the assay. The degree of reproducibility of the result is then determined as a function of assay variables. This reproducibility may be compared to the precision of assay under normal condition to obtain a measure of the ruggedness of the analytical method.

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- Analyst to analyst
- Interday analysis

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, nut deliberate variations in method parameters and provided an indication of its reliability during normal usage.

Robustness tests were originally introduced to avoid problems in liner laboratory studies and to identify the potentially responsible factors

The robustness test can be viewed as a part of method validation that is performed at the end of method development or at the beginning of the validation procedure. The exact position has relatively little influence on how it is performed.

Determination

Robustness was performed by varying

- Change the Flow rate
- Change the Wavelength

Flow rate altered to 1.4ml/min and 1.6ml/min working standard solution were injected as per proposed method and chromatograms were recorded.

Wavelength was altered to 252nm and 256nm and the working standard solution were injected as per proposed method and chromatograms were recorded.

Figure 1. Linearity graph of Losartan Potassium

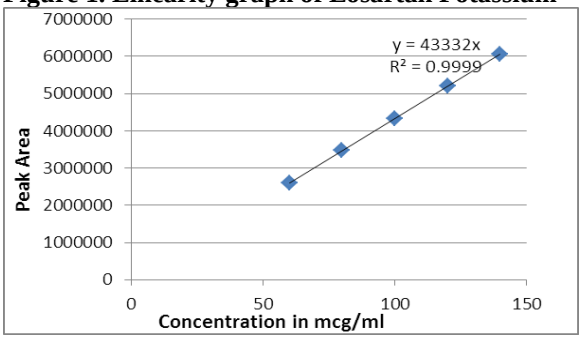

Table 1. Assay

Experiment	Label claim (mg)	Amount of drug found (mg)	Assay %
I	50	50.12	100.24
II	50	50.26	100.52
III	50	49.93	99.86
IV	50	50.04	100.08
V	50	50.09	100.18
VI	50	49.69	99.38

Table 2. Specificity data of Losartan Potassium

Trial	Sample	Area obtained	Average area	% RSD
1.	**Standard**	4276802	42824405	0.2%
		4288079		
2.	**Standard + Placebo**	4217898	4215237	0.1%
		4212576		
3.	**Placebo**	0	0	0%

Table 3. Linearity data of Losartan Potassium

S.No	Concentration in %	Retention time	Area	Mean Area
1.	**60**	7.473	2596890	2594940
		7.480	2592990	
2.	**80**	7.495	3482657	3483602
		7.499	3484547	
3.	**100**	7.516	4331352	4327318
		7.517	4333283	
4.	**120**	7.528	5201144	5201108
		7.528	5201071	
5.	**140**	7.547	6051976	6058400
		7.550	6064824	

Table 4. Recovery data of Losartan Potassium

LEVEL	Amount of drug added (mg)	Amount of standard recovered (mg)*	Recovery* %
I	80	79.3	99.13
II	100	99.96	99.96
III	120	119.0	99.16

*average of three determinations

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Table 5. System precision data of losartan potassium

Parameter	Retention time	Area of Losartan Potassium	Plate count
Trial – 1	7.466	4307646	5565
Trial – 2	7.458	4316756	5720
Trial – 3	7.449	4316995	5708
Trial – 4	7.448	4321683	5680
Trial – 5	7.444	4318342	5750
Average	**7.453**	**4316284.4**	**5685**
S.D	**0.0089**	**5213.6339**	**71.41989**

Table 6. Method precision data of losartan potassium

Trial	Weight taken	Average peak area	Amount present in mg	% Label claim
1.	289.8	4038809	49.33	98.7
2.	299.1	4194156	49.63	99.3
3.	300.4	4203818	49.53	99.1
4.	297.6	4148892	49.35	98.7
5.	301.5	4187739	49.16	98.3
Average				**98.8**
Standard deviation				**0.37**
% Relative standard deviation				**0.4**

Table 7. Ruggedness data of losartan potassium

S.No	Instrument Code	Analyst	Day of Analysis	% Recovery of Losartan Potassium
1.	Water 2695	Raman	Day 1	100.41
2.	Water 2695	Pradeep	Day 1	100.09
3.	Water 2695	Raman	Day 2	99.89
4.	Water 2695	Pradeep	Day 2	98.83

Table 8. Robustness data of losartan potassium

S.No	Wavelength	Flow rate	Average Peak area	Assay in mg	Assay in %
1.	252	1.5	4478982	49.48	99.0
2.	256	1.5	3797553	49.36	98.7
3.	254	1.4	4699900	48.96	97.9
4.	254	1.6	4072890	49.29	98.6

Table 9. Validation Summary

Parameters	Results
System Suitability* **Theoretical Plates (N)** **Asymmetry** **Retention Time**	5715.55 1.124 7.45
Specificity	There is no interaction of placebo in standard and sample peak
Precision* (% RSD) **System Precision (% RSD)** **Method Precision (% RSD)**	 0.0089% 0.4%
Ruggedness* **Analyst to Analyst (% RSD)** **Day to Day (% RSD)**	 0.69%
Robustness* **Changing flow rate (%RSD)** **Changing Wavelength (%RSD)**	 0.47%
Linearity **Range (mcg/ml)** **Correlation co-efficient (R^2)**	 120 mcg/ml – 280 mcg/ml 0.9990
Accuracy (%)	99.13% to 99.96%

*Average of six determinations

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RESULT AND DISCUSSION

Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Losartan Potassium. The assay was linear over the concentration range of 120mcg-280mcg/ml. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the excipients of Losartan Potassium and was found to be 99.16%-99.96%. The ruggedness and robustness %RSD were found within the limits. The system suitability parameters such as theoretical plates, retention time and tailing factor were found to be 5715.55, 7.45 and 1.124 respectively.

CONCLUSION

The developed RP - HPLC method is simple and selective for estimation of Losartan Potassium in tablet dosage form was found to be accurate, rapid and sensitive. The values of coefficient of variance were satisfactory low and recovery was close to 100% indicating reproducibility of the method. The linearity was observed within limit hence method is linear.

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