WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 410-419. Research Article ISSN 2277 – 7105

VALIDATED METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF LOSARTAN POTASIUM AND CHLORTHALIDONE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

Nitin S. Jadhav, K.G. Lalitha*

Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai - 625 020, Tamil Nadu, India.

Article Received on
30 June 2014,

Revised on 25 July 2014,
Accepted on 20 August 2014

***Correspondence for Author**
K.G. Lalitha
Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai - 625 020, Tamil Nadu, India.

ABSTRACT

A new, simple, rapid RP-HPLC method was developed and validated for the simultaneous estimation of Losartan potassium and Chlorthalidone in tablet dosage form. The chromatographic separation was achieved on a Agilent XDB C_{18}(150 x 4.6 mm, 5µm) particle size column was used with PDA detector by using mobile phase containing mixture of 0.02M Potassium dihydrogen orthophosphate (KH_2PO_4) buffer : acetonitrile (70:30 % v/v pH 3.5) was used. The flow rate was 1 ml / min and detection was carried at 254 nm. The retention time for Chlorthalidone and Losartan was found to be 2.718 and 4.848 min. respectively. The method was linear over the concentration range of 12.5 - 75µg/ml for Losartan potassium and 1.55 – 9.35 µg/ml for Chlorthalidone respectively. Limit of detection (LOD) for Losartan potassium and Chlorthalidone was 0.121 µg/ml and 0.0168µg/ml. and Limit of quantitation(LOQ) for Losartan potassium and Chlorthalidone was 0.369µg/ml and 0.0510µg/ml respectively. The proposed method in this study was found to be simple, rapid, precise, accurate, sensitive and applicable for the simultaneous determination of Losartan potassium and Chlorthalidone in combined dosage forms.

KEY WORDS: Losartan potassium, Chlorthalidone, RP-HPLC and ICH guidelines.

INTRODUCTION

Losartan potassium(LOS)[1] is a potassium salt of 2-Butyl-4-chloro-1-[[2¢-(1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol (Fig.1.) It is used as a orally active

non-peptide angiotensin II (Type AT_1) receptor antagonists employed in the management of essential hypertension. Chlorthalidone(CTD)[2] is chemically (RS) 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H- isoindol-1-yl) benzene-1-sulfonamide (Fig.2.) It is a diuretic drug used for the treatment of hypertension.

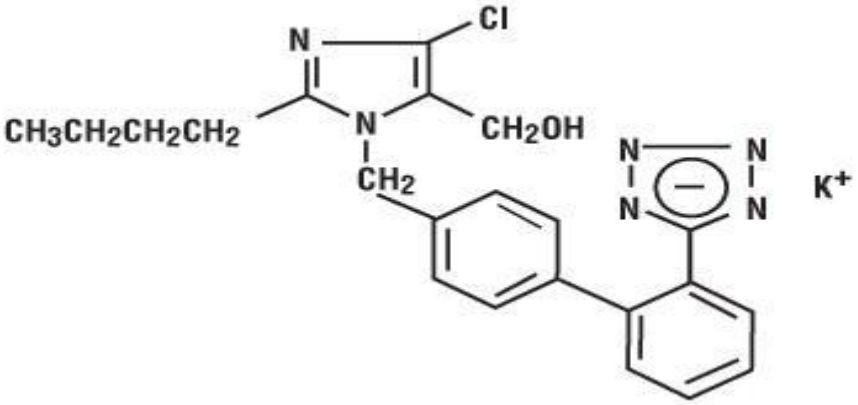

Fig. 1: **Structure of Losartan potassium(LOS)**

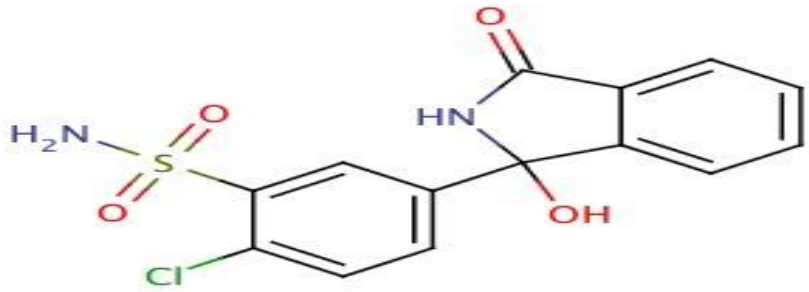

Fig. 2: **Structure of Chlorthalidone (CTD)**

The marketed label claim for losartan potassium and chlorthalidone is 50mg and 6.25mg respectively. Literature survey reveals that Losartan potassium is estimated by using Spectrophometric methods [3-5] and RP-HPLC [6-12] method in combination with some other drugs. Chlorthalidone is estimated by using RP-HPLC [12-15] methods and HPTLC [16] method method in combination with some other drugs. But combination of Losartan potassium and Chlorthalidone cannot be estimated by RP-HPLC method. So, present method aim is to develop simple, rapid and economical RP-HPLC method and validate as per ICH guidelines [17-18] for the simultaneous determination of Losartan Potassium and Chlorthalidone in tablet formulation.

MATERIAL AND METHOD

Chemicals and reagents

Losartan potassium and Chlorthalidone pure samples were obtained from SL Drugs & Pharmaceuticals, Hyderabad, India. The combination of these two drugs in single tablet dosage form was procured from retail pharmacy in the brand name of CTD-L, manufactured by IPCA Pharmaceuticals ltd. HPLC grade Acetonitrile was procured from Rankem India limited, Ankleshwar, India. Methanol and ortho phosphoric acid were procured from Merck specialities Pvt. Ltd, Mumbai and RFC limited, New Delhi respectively. And HPLC grade water was obtained from a Milli – Q water purification system.

Instrumentation and optimized chromatographic conditions

Waters 2695 HPLC with configurations of quaternary pump, Auto sampler, Auto injector, PDA Detector and Empower V.1.2 software was used for data processing. The optimized chromatographic condition were given in Table No 1.0

Table No 1.0

Flow rate	1.0 ml/min
Column	Agilent XDB, C18, 150 x 4.6 mm, 5µ.
Detector wave length	254 nm
Column temperature	30°C
Injection volume	10µL
Run time	8 min
Diluent	Water**:** Acetonitrile (50:50)
Mobile phase	Buffer : acetonitrile (70:30 % v/v pH 3.5)

Preparation of Diluent

The diluent was prepared by mixing 50ml of HPLC water and 50ml of Acetonitrile and the resulting solution was sonicated for 15min.

Preparation of buffer

Accurately weighed about 2.72 gm of Potassium dihydrogen orthophosphate (KH_2PO_4) into a 1000 ml volumetric flask, to that added about 900 ml of HPLC grade water and made final volume with diluent and adjusted the pH-3.5 using ortho Phosphoric acid solution.

Preparation of Standard Solution

Standard stock solution was equivalent to 50 mg of LOS and 6.25mg of CTD was transfer to 25 ml standard flask. The solution was sonicated for 15 minutes and volume made up to 25ml using diluent.

Preparation of Sample for Solution

Twenty tablets weighed and their average weight was calculated. A quantity of powder equivalent to 50mg LOS and 6.25mg of CTD was transferred to 500 ml standard flask; 200ml diluent was added and sonicated for 15 min. Then the volume was made up to the mark with diluent. The solution was filtered through 0.45µ whatman filter paper and 5ml filtrate further diluted into 25ml volumetric flask and final volume was made using diluent. The resulting final clear solution was injected in HPLC in duplicate as per the developed method and the results for the formulations were shown in Table No 2.0

Table No 2.0

Drug	Label claim	Acquired data*	Assay%*
Losartan	50 mg/Tab	50.38 mg/tab	100.77±0.26
Chlorthalidone	6.25 mg/tab	6.31 mg/tab	101.17±0.44s

METHOD VALIDATION

System suitability

System suitability tests were used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. Data from six injections of 10 µL of the working standard solutions were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time. The system suitability results obtained for Losartan and Chlorthalidone is summarized in Table No. 3.0. And the typical Chromatogram of Chlorthalidone and Losartan was shown in Figure 3

Table No 3.0

S.No	Parameter	Losartan	Chlorthalidone
1.	Retention Time	4.63	2.17
2.	USP Theoretical Plate	2622	2640
3.	USP Tailing	1.6	1.9

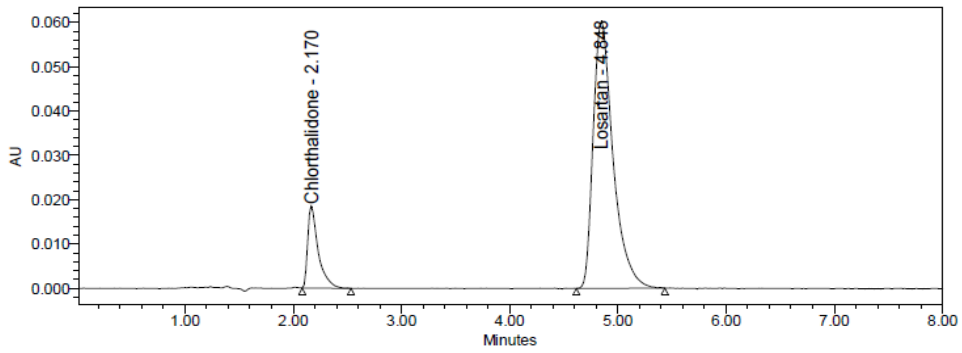

Fig.3: The Typical Chromatogram of Chlorthalidone and Losartan

Linearity

From stock solution diluted to obtain the concentrations ranging from 12.5-75 µg/ml for LOS and 1.55-9.35µg/ml for CTD (80-120% of target level). The 10µl of standard solutions were injected and peak areas were measured. The linearity lie within its specific acceptance criteria was shown in Table No. 4.0 and corresponding calibration curve for losartan and chlorthalidone were shown in Fig. 4.0 and Fig. 5.0 respectively.

Table No. 4.0

Sr.No.	Concentration range for LOS	Peak Area	Concentration range for CTD	Peak Area
1	12.5	753820	1.55	131737
2	25	1535317	3.1	253128
3	37.5	2255254	4.65	367882
4	50	2956937	6.25	485151
5	62.5	3778461	7.80	606678
6	75	4495215	9.35	713679
Slope		**59876X**		**75953X**
Intercept		**8227**		**9435**
Correlation coefficient		**0.9990**		**0.9990**

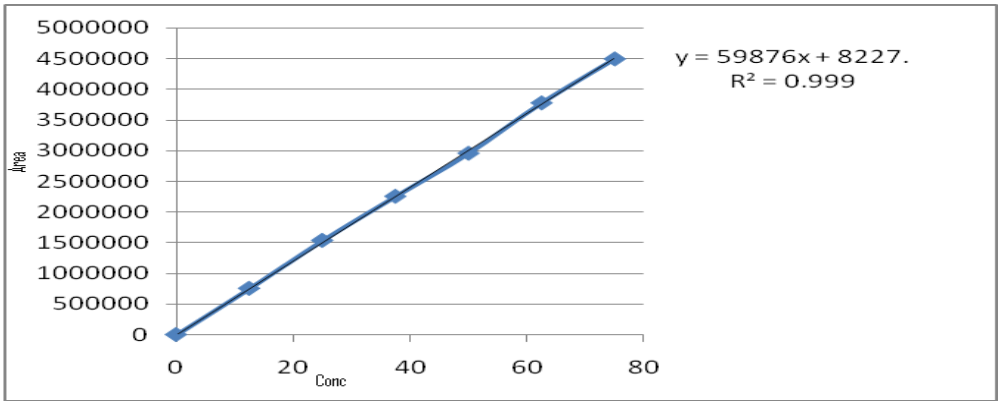

Fig.4: Calibration curve of Losartan potassium

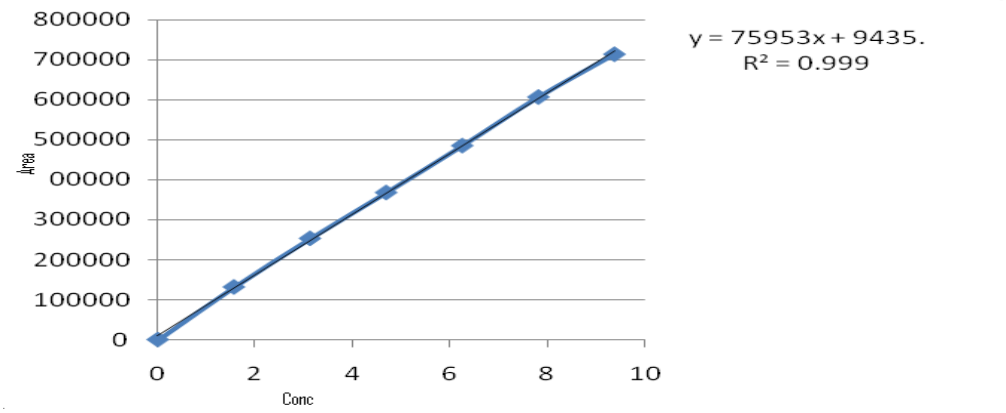

Fig.5: Calibration curve of Chlorthalidone

Accuracy

The accuracy of the method was determined by recovery experiments. The solutions were injected in triplicate in 50%, 100% and 150% concentrations and percentage Recovery was calculated separately for LOS and CTD and summarized in Table No. 5.0 and Table No. 6.0 Respectively.

Table 5.0: Result for Accuracy of Losartan

Sr.No	Chlorthalidone	Amount Recoverd	%Recovery	Avarage Recovery	% RSD
1	50%	25.31 25.19 25.38	101.27 100.77 101.53	101.19	0.381
2	100%	50.34 50.28 50.53	100.68 100.56 101.07	100.77	0.264
3	150%	75.35 75.61 75.28	100.47 100.82 100.37	100.55	0.234

Table 6.0: Result for Accuracy of Chlorthalidone

Sr.No	Chlorthalidone	Amount Recoverd	%Recovery	Average Recovery	% RSD
1	50%	3.12 3.16 3.14	100.22 100.43 100.38	100.34	0.109
2	100%	6.29 6.34 6.32	100.69 101.56 101.26	101.17	0.441
3	150%	9.28 9.28 9.42	99.06 99.00 100.57	99.54	0.893

Precision

It is very important that the method developed should be precise. Six replicates of the sample prepared from the tablet were injected and assay was calculated to measure the repeatability of retention times and peak area of standard and sample. The percentage % RSD values for was calculated and shown in Table No. 7.0

Table 7.0: Method precision results

Drug	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Average	%RSD
Losartan	100.61%	100.65%	100.84%	100.03%	99.69%	99.48%	100.22 %	0.50
Chlorthalidonee	100.38%	100.36%	100.52%	100.35%	100.08%	100.66%	100.39%	0.20

Limit of detection and Limit of quantization

Limits of detection (LOD) and limit of quantization (LOQ) were estimated from the signal-to-noise ratio. The detection limit was determined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantization limit was determined as the lowest concentration level that provided a peak area with signal-to-noise 10. The results for LOD and LOQ was shown in Table No. 8.0

Table 8.0: Results of LOD and LOQ for Losartan potassium and Chlorthalidone

Content	LOD(µg/ml)	LOQ(µg/ml)
Losartan potassium	0.121	0.369
Chlorthalidone	0.0168	0.0510

Specificity

The Specificity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis. Excipients for each tablet were mixed in order to prepare a placebo, and solutions were prepared by following the procedure described in the section on sample preparation. The tablets excipients did not interfere with the method.

Robustness

The robustness of the developed method was established in differently and deliberately by varied chromatographic conditions e.g. Change in flow rate and detection wavelength. The robustness was checked by changing the flow rate 0.9 and 1.1 ml/min and the mobile phase (±2) and column temperature (±5) in losartan potassium and Chlorthalidone respectively. The corresponding data for LOS and CTD was shown in Table No 9.0 & 10.0 respectively.

Table 9.0: Losartan potassium % RSD by change in flow rates, by change in mobile phase & by change in column temperature

By Change In	Average Area	%RSD
Flow rate 0.9 ml/min	3248385	0.90
Flow rate 1.1 ml/min	2623776	0.10
Mobile phase -2 nm	2924800	0.90
Mobile phase +2 nm	29427501	1.6
Column temp -5ºc	2977975	1.6
Column temp +5ºc	2894785	0.30

Table 10.0: Chlorthalidone % RSD by change in flow rates, by change in mobile phase & by change in column temperature

By Change In	Average Area	%RSD
Flow rate 0.9 ml/min	518776	0.90
Flow rate 1.1 ml/min	418863	0.10
Mobile phase -2 nm	484921	0.50
Mobile phase +2 nm	469274	1.6
Column temp -5ºc	472663	1.6
Column temp +5ºc	459655	0.40

RESULTS AND DISCUSSION

From the optimized method and above observations the present work is successfully carried out for the development and validation of Losartan potassium and Chlorthalidone in tablet dosage form and found to be suitable for the simultaneous estimation by using RP-HPLC method. The developed method was validated as per ICH guidelines for accuracy, linearity, precision, specificity, LOD & LOQ and robustness.

CONCLUSION

It is concluded from the above study that the current method is fast, reproducible, simple and economical. By adopting this method one can elute both two drugs in 8 minutes. Hence this method is definitely time saving to enable the simultaneous estimation of Losartan potassium and Chlorthalidone in different formulations. The proposed method is found to be accurate, precise, linear, specific and robustness.

ACKNOWLEDGEMENT

The authors are thankful to ultra college of pharmacy, Madurai for providing necessary facilities for carrying out this work.

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