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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND PERINDOPRIL ERBUMINE IN PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Losartan Potassium and Perindopril erbumine in pharmaceutical dosage form. The mobile phase consisted of Acetonitrile: pot.dihydrogen phosphate buffer (0.1% Trifluoroacetic acid is used as apeak sharpener) in the ratio of 40:60 delivered at a flow rate of 0.8 mL / min and wavelength of detection at 217 nm. The retention times of were Losartan Potassium and Perindopril erbumine 5.87 min and 3.61 min respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantification. The method was found to be linear in the range of 25-150µg/ml and 1-6 µg/ml for Losartan potassium and Perindopril erbumine respectively. The coefficient of variance for both the drug was more than 0.999.The proposed method can be used for determination of these drugs in combined dosage forms.
Key words: Losartan, Perindopril, RP- HPLC, and Validation.

INTRODUCTION

Losartan potassium (LOS) a potassium salt of 2-Butyl- 4-chloro-1-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, represents the first of a new class of orally active non-peptide angiotensin II (Type AT1) receptor antagonists employed in the management of essential hypertension[1].Perindopril erbumine (BP, 2007), Chemically (2S, 3αS, 7αS)-1-[(S)-N-[(S)-1-Carboxy-butyl] alanyl] hexahydro-2-indoline carboxylic acid,1-ethyl ester, compound with tert-butylamine. Perindopril erbumine is an anti-hypertensive agent and prodrug for perindoprilat, which inhibits ACE in human subjects and animals. Perindopril Erbumine is one of the non-peptide Angiotensin II receptor antagonists, and is used for the treatment of patients with hypertension and symptomatic heart failure[2]. The combined oral administration of perindopril with losartan has been found to be more effective than either of the drugs alone in the treatment of hypertension. Structures of Losartan and Perindopril erbumine are shown in Fig **1**.

Upon detailed literature survey it was found that, individually and combined with some other drugs have been analyzed by many methods i.e. one Spectrophotometric with LC method[3],one stability indicating RP-HPLC method, [4] Nine RP-HPLC methods has been reported with other drugs combination[5-13]. To the best of our knowledge, only one RP-HPLC method has been described for simultaneous estimation of both the drugs in tablets.[14] The present work describes the simple, accurate, precise, sensitive RP-HPLC method for the determination of Losartan Potassium and Perindopril erbumine in combination. The method was validated as per the ICH guidelines.[15-16.]

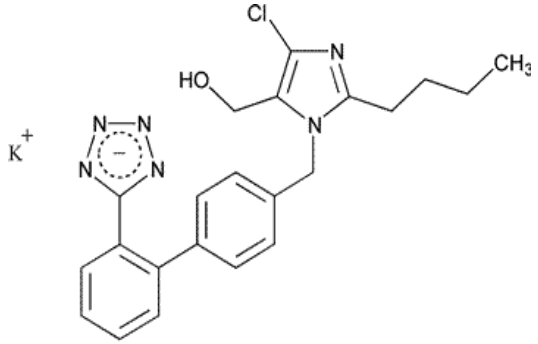

Fig.no.1(a) Chemical structure Losartan potassium

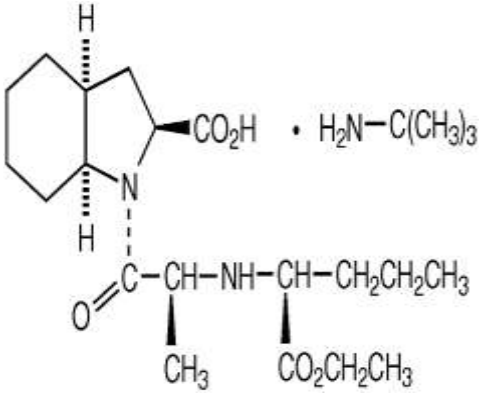

Fig.no.1 (b) Chemical structure of Perindopril erbumine

MATERIALS AND METHODS

Instruments

High performance liquid chromatography including A Quaternary Gradient WATERS HPLC e2695 with QCL-034 Software with UV-Visible Detector (WATERS 2489), PUMP (LC-20AT) and (LC-20ATvp), UV-Visible Spectrophotometer (UV-2450 SHIMADZU). Analytical Balance (SARTORIUS), P^H Analyzer (POLMON), Triple Quartz Distillation Unit (BOROSIL) and Ultra Sonicator (BIOTECHNICS) were used.

Chemicals and Reagents

Reference standard of losartan potassium and perindopril erbumine were contain purity 99.92% and 99.45 % respectively. Reference standard of losartan potassium (99.92 %) and perindopril erbumine (99.45 %) were gifted by Ranbaxy pharmaceutical Ltd., Dr.Reddy's Labs pvt. Ltd., respectively. The tablet formulation procured from local market label claim for losartan potassium and perindopril erbumine were 50 mg and 2 mg respectively. Acetonitrile HPLC grade (Rankem, Mumbai, India), water HPLC grade and *o*-phosphoric acid, TrifluoroaceticAcid ARgrade reagents were used. Stationary phase Nucleosil C18, 100 mm x 4.6 mm, was used.

Selection of Wavelength

UV scan of Losartan Potassium and Perindopril erbumine the was done individually and both Were over lapped upon each other to get the required wavelength .The wavelength of 217 nm was found to be effective in determination of both the drugs at a time.

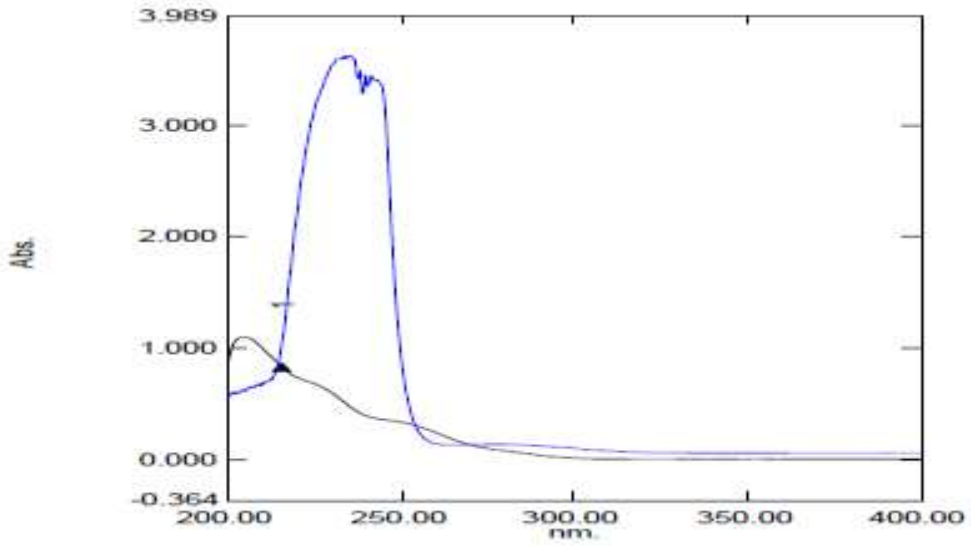

Figure-2: UV Spectra of Losartan potassium and Perindopril erbumine

Standard stock Preparation:

Weighed and transferred accurately about 50 mg losartan potassium and 2mg perindopril erbumine of Working Standards into a 10 ml clean dry volumetric flask, then dissolved and make up this volume with methanol, finally sonic at for 5 minutes.

Diluted Standard:

Pipette out 1ml from the standard stock solution, into a 50 ml clean dry volumetric flask, and dilute to the mark with 50 ml of diluent.

Sample preparation:

Weigh and powdered about twenty tablets in a neat clean and dry motor and pestle .weighed and transferred accurately about 50mg and 2 mg drugs in the tablet powder into 10 ml clean dry volumetric flask, add about10 ml methanol, sonicate for 15 minutes, and dilute to volume with diluent. Filter the solution through the what's man filter paper, from the filtrate pipette out 1ml of sample solution into a 25ml volumetric flask, make up the volume with diluent (mobile phase).

RESULTS AND DISCUSSION:

1) Linearity

Calibration graphs were constructed by plotting peak area Vs concentration of losartan and perindopril the regression equation were calculated. Linear correlation was obtained between peak areas and concentration of Losartan potassium and Perindopril erbumine in the range of 25-150µg/ml and 1-6 µg/ml respectively for both the drugs. Data of the regression analysis are summarized in Table 1

2) Accuracy

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in Table 2.

3) Method precision (Repeatability):

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. The result of precision study is presented in Table 3.

4) System precision :(Reproducibility):

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). Reproducibility is assessed by means of an inter-laboratory trial. The system precision was checked by repeatedly injecting (n = 6) mixed standard solution of Losartan and perindopril erbumine. The result of system precision is presented in table 4.

5) System suitability

According to the ICH, system suitability tests are an integral part of chromatographicmethods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Data of the analysis are summarized in Table 5.1.

6). Limit of detection (LOD) and limit of quantitation (LOQ):

The LOD with signal to noise (S/N) ratio of 3:1 and LOQ with (S/N) ratio of 10:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines[2].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

LOD values for Losartan and Perindopril were found to be 0.1682 and 0.132µg/ml respectively. LOQ values for Losartan and Perindopril were found to be 0.462 and 0.413 µg/ml respectively. Data of the analysis are summarized in Table 6.1.

7). Assay:

The proposed validated method was successfully applied to determine Losartan Potassium and Perindopril Erbumine in tablet dosage form. The result obtained for Losartan Potassium and Perindopril Erbumine were comparable with corresponding labelled amounts.

Table No: 1.1: Losartan potassium

S.NO	% of Test	Concentration (µg/ml)	Area
1	25	25	448535
2	50	50	920528
3	75	75	1363160
4	100	100	1822865
5	125	125	2272774
6	150	150	2710100

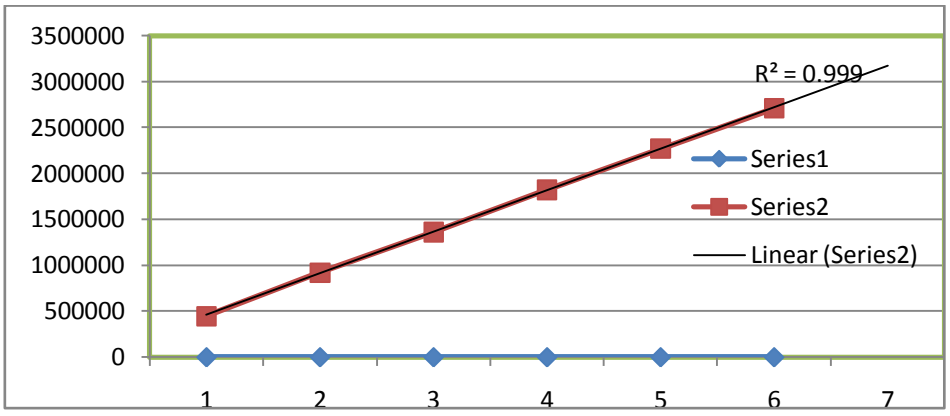

Fig. no.3 Linearity graph of Losartan potassium.

Table no: 1.2 Perindopril erbumine:

S.NO	% of test	Concentration(µg/ml)	Area
1	25	1.00	44776
2	50	2.00	91847
3	75	3.00	134995
4	100	4.00	182187
5	125	5.00	226837
6	150	6.00	267625

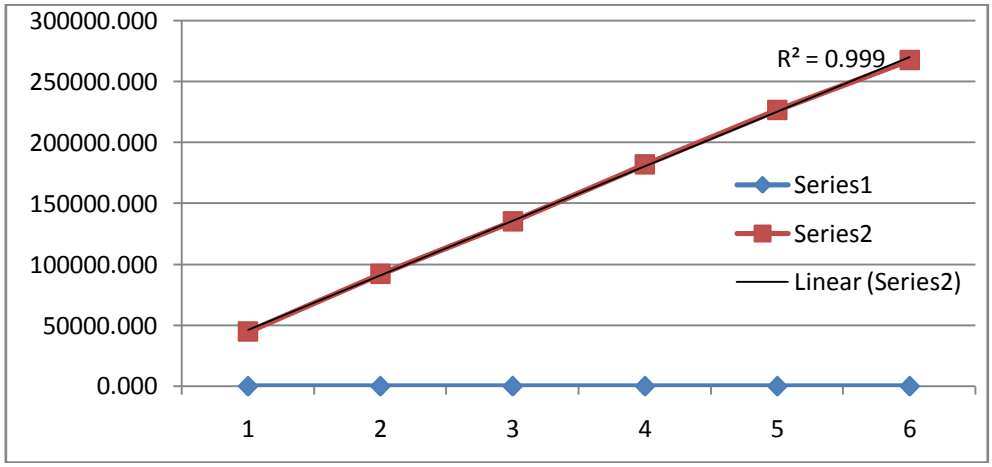

Fig. no. 4 Linearity graph of Perindopril erbumine

Table no- 2.1 : Losartan potassium

S.no	Spike Level	µg/ml added	µg/ml found	%recovery	mean % recovery
1	80%	80	79.52	99.99	
2	80%	80	80.01	100.01	99.42%
3	80%	80	79.56	99.99	
1	100%	100	99.04	99.64	
2	100%	100	99.04	98.84	99.02%
3	100%	100	99.03	99.86	
1	120%	120	119.9	100.00	
2	120%	120	119.9	99.99	99.15%
3	120%	120	119.9	99.99	

Table no: 2.2 Perindopril Erbumine

S.no	**Spike Level**	**µg/ml added**	**µg/ml found**	**%recovery**	**Mean % recovery**
1	80%	80	79.70	99.62	
2	80%	80	79.69	99.63	99.62%
3	80%	80	79.72	99.62	
1	100%	100	99.23	99.24	
2	100%	100	99.23	99.23	99.23%
3	100%	100	99.23	99.23	
1	120%	120	119.31	99.42	
2	120%	120	119.30	99.41	99.42%
3	120%	120	119.35	99.42	

Table no: 3.1 Losartan potassium

S.No	**Retention Time(min)**	**Area**
1	5.879	1826541
2	5.881	1836524
3	5.891	1824315
4	5.886	1813124
5	5.877	1829987
6	5.875	1836548
Average	5.881	1827840
S.D	0.006	8792.52
%R.S.D	0.10	0.48

Table no: 3.2 Perindopril erbumine

S.No	Retention Time(min)	Area
1	3.608	182154
2	3.609	181965
3	3.612	180356
4	3.611	182689
5	3.599	181935
6	3.602	180264
Average	3.607	181561
S.D	0.0052	1006.1
%R.S.D	0.1440	0.554

Table no: 4.1: Losartan potassium

S.No	Retention Time(min)	Area
1	5.888	1823429
2	5.886	1819635
3	5.892	1826653
4	5.893	1819460
5	5.896	1812568
6	5.897	1827584
Average	5.892	1821555
S.D	0.004	5562.23
%R.S.D	0.07	0.31

Table no: 4.2: Perindopril erbumine

S.No	Retention Time(min)	Area
1	3.603	181765
2	3.601	181093
3	3.604	181157
4	3.605	180842
5	3.606	180476
6	3.607	181286
Average	3.604	181103
S.D	0.0022	432.7
%R.S.D	0.0599	0.239

Table No. 5.1

Parameter	Acceptance criteria	Observed value
1.Theoritical plates		
Losartan Potassium		9959.49
Perindopril erbumine	(not less than 3000)	6634.58
2.Tailing factor		
Losartan Potassium	(not less than 2)	1.05
Perindopril erbumine		1.11
3.Repeatability		
Losartan Potassium	(RSD <1% for N>5)	0.48
Perindopril erbumine		0.554
4.Resolution(Rs)	(Rs>2)	10.93

Table no: 6.1

Parameter	LOSARTAN	PERINDOPRIL
LOD	0.1682µg/ml	0.132µg/ml
LOQ	0.462 µg/ml	0.413 µg/ml

Table no: 7

Drug	% Assay	Amount present
Losartan Potassium	98.91	49.46mg/tab
Perindopril Erbumine	99.00	1.98 mg/tab

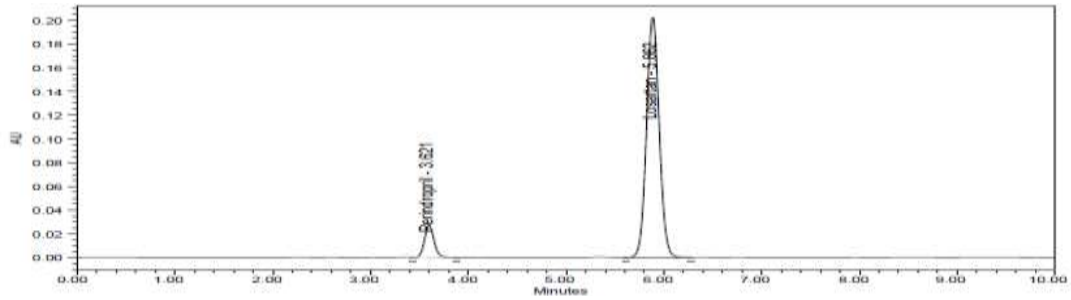

Fig: 5. Typical chromatogram of Losartan potassium and Perindopril erbumine (standard)

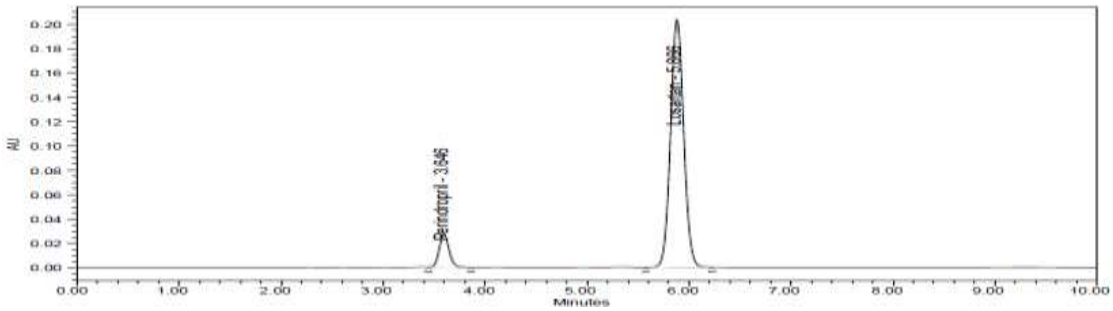

Fig: 6.Typical chromatogram of Losartan potassium and Perindopril erbumine (test)

CONCLUSION

The proposed method has advantage of simplicity and convenience for the separation and quantitation of losartan potassium and perindopril erbumine in the combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of losartan potassium and perindopril erbumine in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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REFERANCES

1. Indian Pharmacopoeia, CBS publisher, Volume-2, **1996**, 469-470.
2. Anonymous. British Pharmacopoeia. **2007**. Vol II, International Edition, Controller of HMSO: London; 1607.
3. ErK Singvi I, Anju A. Comparision of spectrophotometric and LC method for the determination of Perindopril and Indapamide in pharmaceutical formulations, *Journal of Pharmaceutical and Biomedical Analysis*, **2001**; 26:43-52.
4. Deanne L.H, Jennifer F. Development and validation of stability indicating HPLC method for the simultaneous determination of Losartan Potassium and Hydrochlorothiazide and their degradation products, *Journal of pharmaceutical and biomedical analysis*, **2002;** 30:747-760.
5. Suhagia B.N., Shah,R.R., Patel,D.M. Development of RP-HPLC method for evaluating losartan potassium and hydrochlorthizide tablets**,** *Indian Journal of Pharmaceutical Science* **2005;**67:37-42.

6. SMD Riyaz, Vasanth PM, Ramesh M, Ramesh R, Ramesh T. A Sensitive RP-HPLC Method Development and Validation for the simultaneous estimation of Perindopril Erbumine and Amlodipine Besilate in Tablet Dosage Form, *International Journal of Chemical and Life sciences,* **2012;** 01 (02):1033-1038.
7. Ramya Gavini, S.B.puranik, G.V.S.Kumar, K.A.Kumar, K.A.Kumar. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Amlodipine and Losartan in Bulk and Tablet Dosage Formulation,*International Research Journal of Pharmacy,* **2012;** 3(11):92-95.
8. Mohit G Dewani, Kailash G Bothara, Ashwini R Madgulkar and Mrinalini C Damle. Simultaneous Estimation of Perindopril Erbumine and Indapamide in bulk drug and Tablet dosage form by HPTLC, *Pharmacie Globale (IJCP),* **2011;** 1 (01):1-4.
9. Juddy Joseph, Blessen Philip, Dr. M. Sundarapandian. Method Development and Validation for Simultaneous estimation of Perindopril Erbumine and Indapamide by RP-HPLC in Pharmaceutical Dosage Forms,*International Journal of Pharmacy and Pharmaceutical Sciences,* **2011;** 3 (Suppl 4):288-293.
10. Ankit. B. Chaudhary, Rakesh. K. patel, Sunita. A. Chaudhary. Determination of Losartan Potassium and Perindopril Erbumine in Tablet Formulations by Reversed-Phase HPLC, *International Journal of ChemTech Research*, **2010;** 2(2):141-1146.
11. Srinivasa Rao K, Srinivas K. RP-HPLC method for the determination of losartan potassium and ramipril in combined dosage form, *Indian J Pharm Sci*, **2010;** 72(1):1-4.
12. Deanne Hetzog L, Jennifer Finnegan McCafferty, Xueguang fang, Jeffrey Tyrrell R, Robert Reed A. Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide and their degradation products , *J Pharm Biomed Anal*, **2002;** 30:747-60.
13. Kavitha J, Muralidharan S.Development And Validation Of New Method For Atenolol, Hydrochlorothiazide And Losartan Potassium By RP-HPC, *Int J ChemTech Res,* **2010;**2(2):880-894.
14. A.I. Patel, C.K.Oza, J.P. Prajapati, A.J.Vyas, P. Mehta **.**RP-HPLC method for the determination of Losartan Potassium and Perindopril Erbumine in combined tablet dosage form, *International Journal of Pharma and Bio Sciences*, **2011;** 2(1):709-715.
15. Lloyd R. Synder, Joseph J. Kirkland, Joseph L. Glajesh, Practical HPLC Method Development, 2nd Edition, **1997**,1-14.
16. ICH-Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for HumanUse, Geneva, Switzerland, **1996.**