ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

RP-HPLC METHOD FOR THE DETERMINATION OF LOSARTAN POTASSIUM AND PERINDOPRIL ERBUMINE IN COMBINED TABLET DOSAGE FORM.

Corresponding Author

A. I. PATEL

zydus research center, ahmedabad. India.

Co Authors

C.K.OZA[1], J.P. PRAJAPATI[2], A.J.VYAS[3]AND P. MEHTA[1]

[1]Institute of pharmacy, Nirma university of science & technology, Ahmedabad. India.
[2]Zydus research center, ahmedabad. India.
[3]BKmody govt. Pharmacy college rajkot, india.

ABSTRACT

A simple, fast, and precise reverse phase high performance liquid chromatographic method was developed and validated for the simultaneous estimation of losartan potassium and perindopril erbumine in its tablet form. The HPLC method involved by using HiQSil-C-18W ODS, (250 mm × 4.5 mm i.d.), 5 µm column and mobile phase was ACN: water in proportion of 50:50 v/v, pH adjusted to 3.2 ± 0.1 with 1 % o-phosphoric acid. The flow rate was 1.0 mLmin^{-1} and effluent was monitored at 210 nm. The retention time of losartan potassium and perindopril erbumine were eluted at 6.7 min and 4.5 min respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection. The method was found to be linear in the range of 2-18 µgmL^{-1} for both the drug. The coefficient of variance for both the drug was more than 0.999. The mean percentage recovery was found to be 98.40 % for losartan potassium and 97.50 % for perindopril erbumine. The limits of quantification of losartan potassium and perindopril erbumine were found to be 0.109µgmL^{-1} and 0.041µgmL^{-1}. The method has been successfully applied for determination of losartan potassium and perindopril erbumine in combined dosage form.

ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

KEYWORDS

Perindopril erbumine, Losartan potassium, Simultaneous estimation, RP-HPLC.

INTRODUCTION

Losartan potassium (LST) is an anti-hypertensive drug[1]. It is an angiotensinogen II receptor antagonist that acts by blocking the action of rennin-angiotensinogen-aldosterone system. Several methods such as HPTLC[2], HPLC[3] and LC/tandem mass spectrometry[4] have been reported in the literature review. Perindopril erbumine (PER) is also an anti-hypertensive drug which is an angiotensinogen converting enzyme inhibitors. Several methods such as Spectrophotometric[6], RP-HPLC[7] have been reported in literature. In European pharmacopoeia, HPLC method is reported for estimation of impurities in reference standard of perindopril erbumine. Literature survey reveals that so far none of the method is reported for the simultaneous estimation of losartan potassium and perindopril erbumine. Combined dosage form of losartan potassium and perindopril erbumine is available in form of tablet formulation in indian market. For the purpose of same RP-HPLC method was developed for simultaneous determination of losartan potassium and perindopril erbumine from solid pharmaceutical dosage form, which is simple, precise, accurate, and rapid.

MATERIALS AND METHODS

Instruments

High performance liquid chromatography including JASCO PU-2080 Plus pump equipped with universal injector (Rheodyne) with injection volume 20 µL, JASCO UV-2075 Plus UV-visible detector. Analytical balance (Keroy Pvt. Ltd.), pH meter (Analab scientific instrument Ltd.), Ultra sonicator (Trans-O-Sonic Ltd.) were used.

Chemicals and Reagents

Reference standard of losartan potassium and perindopril erbumine were contain purity 99.85 % and 99.45 % respectively. Reference standard of losartan potassium (99.85 %) and perindopril erbumine (99.45 %) were gifted by torrent pharmaceutical Ltd., glenmark pharmaceutical 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 (CDH, Ankleshwar, India), water HPLC grade and *o*-phosphoric acid ARgrade reagent (CDH, Ankleshwar, India) were used. Stationary phase HiQSil-C-18W, 5 µm, (250 mm× 4.5 mm i.d.) was used.

Preparation of standard stock solution

Each 10 mg of losartan potassium and perindopril erbumine was dissolved in 50 mL ACN separately in 100 mL volumetric flask, by sonicating for 15 min. Make up the volume up to the mark with ACN.

Preparation of sample solution

Twenty tablets were weighted and powered properly. A quantity of tablet powder equivalent to 1 mg of perindopril erbumine and 25 mg of losartan potassium was weighted accurately and transferred to 100 mL volumetric flask with accurate weighted 24 mg reference standard of perindopril erbumine dissolved the content in about 50 mL ACN by sonicating for about 20 min in ultrasonicating bath. The volume was made up to the mark with ACN. The solution was filter through the Whatman filter paper No. 41 and suitably diluted with mobile phase to obtain a mixture of suitable concentration.

Calibration and precision of assay

Aliquots of losartan potassium and perindopril erbumine standard stock solution were taken in the 10 mL volumetric flask and diluted up to the mark with mobile phase such that the final concentration each of losartan potassium and perindopril erbumine in mixed standard were in the range of 2-18 μgmL^{-1}. Evaluations of two drugs were done with UV detection with 210 nm. Peak areas were recorded for all the peaks. The plots of peak area versus the respective concentration of losartan potassium and perindopril erbumine were found to be linear.

Validation of method

The method was validated for accuracy, precision, detection limit, quantification limit.

Accuracy:

Accuracy of the method was evaluated by the determination of recovery of LST and PER in duplicated at three levels such as 80 %, 100 % and 120 % of the method concentration.

Intraday and Inter day precision:

The intra day and inter day precision study of LST and PER was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days(freshly prepared) for 3 different concentrations of LST (6, 12, 18 μgmL^{-1}) and PER(6, 12, 18 μgmL^{-1}).The standard deviation, and coefficient of variation was calculated.

Limit of detection and Limit of quantification:

Calibration curve was repeated 3 times and the standard deviation (SD) of the intercepts (response) was calculated. Then LOD and LOQ were measured by using mathematical expressions.

LOD = 3.3*N/S

LOQ = 10*N/S

Where

N is SD of response.

S is slope is the corresponding calibration curve.

RESULTS

The calibration curve for LST was obtained by plotting the peak area of LST versus concentration of LST over the range of 2-18 μgmL^{-1}, and it was found to be linear with r = 0.9975. Similarly, the calibration curve for PER was obtained over the range of 2-18 μgmL^{-1} and was found to be linear with r= 0.9963. The data of regression analysis of the calibration curves are shown in [Table - 1], [Table - 2]. The recoveries of LST and PER were found to be in the range of 98.93 - 101.16 % and 97.82-101.30 %, respectively. The validation parameters are summarized in [Table -4]. The proposed liquid chromatographic method was applied to the determination of LST and PER in their combined dosage forms. The results for LST and PER were comparable with the corresponding labeled amounts [Table - 5].

TABLE: 1
CALIBRATION DATA FOR LST.

Concentration of LST (μgmL^{-})	Peak area Mean*± SD	% RSD
2	555506.66 ± 550.80	0.09
4	757154.29 ± 994.52	0.13
6	1024278.63 ± 1637.42	0.15
8	1301323.88 ± 950.39	0.07
10	1571933.52 ± 1000.06	0.06
12	1792204.25 ± 208.20	0.12
14	2056561.21 ± 1001.66	0.04
16	2276524.90 ± 1527.12	0.06
18	2450330.50 ± 1437.99	0.18

**Indicates mean of the triplet*

TABLE: 2
CALIBRATION DATA FOR PER.

Concentration of PER (μgmL^{-})	Peak area mean* ± SD	% RSD
2	95515.28 ± 321.98	0.33
4	124811.10 ± 155.67	0.12
6	170106.50 ± 829.68	0.49
8	219818.40 ± 197.39	0.08
10	285307.40 ± 302.18	0.10
12	332175.00 ± 840.38	0.25
14	380553.60 ± 862.13	0.22
16	420537.40 ± 601.78	0.14
18	479009.30 ± 810.60	0.16

**** Indicates mean of the triplet***

TABLE: 3
OPTIMIZED CHROMATOGRAPHIC CONDITION

Chromatographic condition	Result
Mobile phase	Acetonitrile: water (50:50 v/v) at pH 3.2 using orthophosphoric acid as acidifier.
Column	**:** HiQ Sil-C-18W ODS (5 µm, 4.6 x 250 mm)
Flow rate	1 $mLmin^{-1}$
Detection wavelength	210 nm
Injection volume	20 µL
Run time	7 min

TABLE: 4
VALIDATIONS AND SYSTEM SUITABILITY TEST PARAMETERS.

System Suitability Parameters	Proposed Method	
	LST	PER
Retention time* (min)	6.7	4.5
Tailing factor	1.54	1.43
Theoretical plates	6482	3482
Linearity Range (μgmL^{-1})	2-18	2-18
%recovery	98.40	97.50
Resolution factor	7.05	-
Limit of detection (µgmL-1)	0.035	0.013
Limit of quantification (µgmL-1)	0.109	0.041
Correlation coefficient	0.998	0.996

TABLE: 5
ANALYSIS OF MARKETED FORMULATION.

Formulations	Label claim (mg)		Amount found (mg)		% Recovery* ± SD	
	LST	PER	LST	PER	LST	PER
ADPACE	50	2	49.20	1.95	98.40 ± 1.23	97.50 ± 1.0

Indicates mean of the triplet.

TABLE: 6
SPIKED CONCENTRATION OF DRUGS TO THE PREANALYSED DOSAGE FORM.

Drugs	Concentration	Intraday variation	Inter day variation	COV	
				Intraday	Inter day
LST	6	1024621.37	479207.75	0.05	0.02
	12	1795843.38	1793405.23	0.08	0.07
	18	2452324.84	2455648.65	0.09	0.04
PER	6	170743.80	1025698.32	0.71	0.04
	12	332188.56	1793405.23	0.02	0.20
	18	479207.75	2455648.65	0.03	0.31

TABLE: 6
INTRADAY AND INTERDAY VARIATION S.

Drugs	Concentration before spiking (µg/ml)	Reference standard added (µg/ml)	Concentration after spiking (µg/ml)	Amount of drugs found	% Recovery	Mean % recovery
LST	8	6.4	14.4	14.2	98.61	
	8	8.0	16.0	15.8	98.75	
	8	9.6	17.6	17.1	97.15	98.17
PER	8	6.4	14.4	14.3	99.30	
	8	8.0	16.0	15.8	98.75	
	8	9.6	17.6	17.3	98.29	98.78

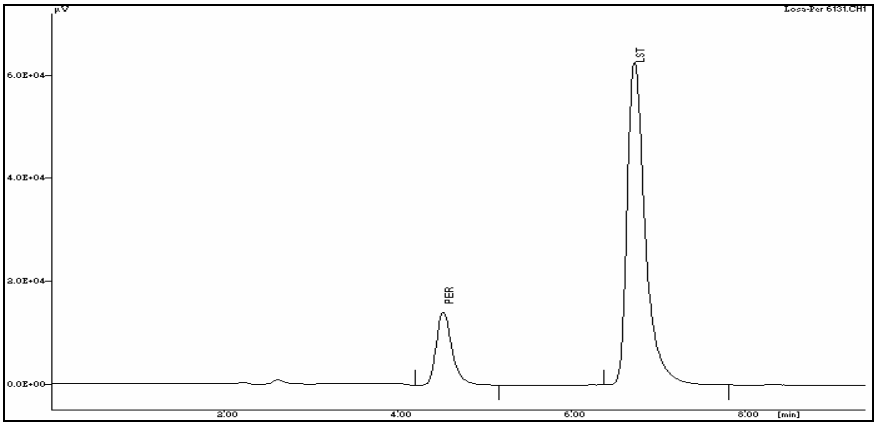

Fig.1
Typical chromatograph of test sample (LST:Losartan potassium, PER: Perindopril erbumine).

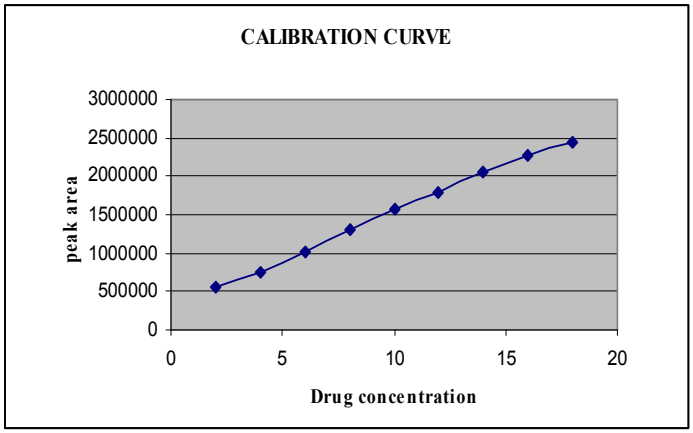

Fig: 2
Calibration curve of LST.

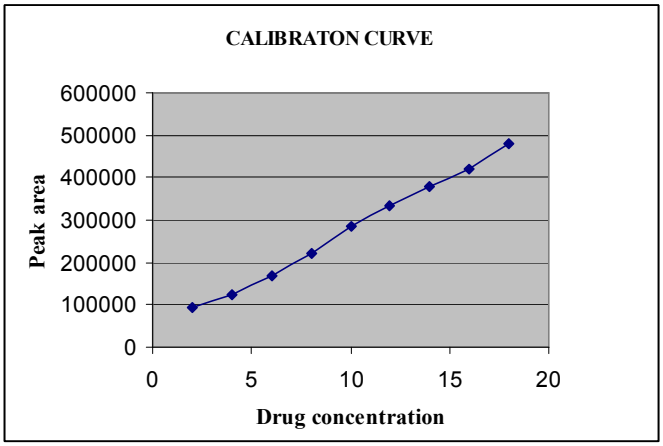

Fig: 3
Calibration curve of PER

DISCUSSION

The result obtain by proposed method were in good agreement with the label claim. The additives and excipients present in the tablet did not interference. The value of standard deviation and %RSD values were satisfactorily low that indicate accuracy and reproducibility of this method. Hence it can used for the routine analysis of simultaneous determination of LST and PER in pharmaceutical formulation.

High performance liquid chromatography is suitable technique for the reliable analysis of commercial formulation containing of LST and PER. Percentage of recovery from the sample show that the method is free from any interference because of excipients. Thus the proposed method for the determination of LST and PER is accurate. So this method can be used for routine quality control analysis of pharmaceutical formulation.

ACKNOWLEDGEMENTS

The authors greatly acknowledge to Torrent pharmaceutical Ltd. and Glenmark pharmaceutical Pvt. Ltd, for providing the gift sample of losartan potassium and Perindopril erbumine. The authors thank Nirma University for carrying out this work.

REFERENCES

1. Indian Pharmacopoeia, CBS publisher, Volume-2, 1996, p.469-470.
2. 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,p.37-42.
3. Deanne L.H, Jennifer F, Development and validation of stability indicatingHPLC method for the simultaneous determination of losartan potassium ,hydrochlorthizide and their degradation products, Journal of pharmaceutical and boimedical analysis 2002;30, p.747-760.
4. Seburg R.A., Ballard, J.M., Hwang T.L., Sullivan C.M., Photosensitized degradation of losartan potassium in an extemporaneous suspension formulation, Journal of Pharmaceutical and Biomedical Analysis 2006;42, p.411-422.
5. Raman N.V.V.S.S., Reddy R.K., Reddy R.M., RP-HPLC of perindopril erbumine and its isomeric impurities ,Indian Drugs 2007;44, p.290-294.
6. Erk N, Comparison of spectrophotometric and an LC method for the determination perindopril and indapamide in pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis 2001; 26, p.43-52.
7. Harlikar J.N. and Amalani A.M., Separation and quantification of stereoisomer (impurity of perindopril tert-butyl amine from pharmaceutical formulations and active pharmaceutical ingredient (API) using reverse phase liquid chromatography, Indian drug 2004;41,p.741-744.