INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ATROVASTATIN AND LOSARTAN IN PURE AND TABLET FORMULATIONS

R. Vijayalakshmi, P. Kalyani, P. Sandya and MD. Dhanaraju*

Research Lab, GIET School of Pharmacy, NH-5 Chaitanya Knowledge City,

Rajahmundry, Andhra Pradesh, India.

ABSTRACT

The combination of Atorvastatin and Losartan was used in the management of Hypertension. The present work was focused on the development of RP-HPLC method for the estimation of Atorvastatin and Losartan in binary mixtures by internal standard method. The separation was performed on LUNA C18 column at 238 nm by isocratic elution using Telmisartan as internal standard. Methanol and phosphate buffer (pH 6.8) in the ratio of 80:20 with a flow rate of 1ml/min at ambient temperature was found to be suitable for chromatographic separation. Atorvastatin and Losartan exhibits linearity in the range of 60-120 and 96-180 µg/ml respectively. The proposed method evidenced the absence of chromatographic interference by pharmaceutical excipients. Separation of the mixtures was proved to be good from the resolution of the peaks. Validation and recovery studies were performed and the results proved the method is suitable for routine analysis.

Keywords: Atorvastatin, Losartan, RP-HPLC, internal standard, Telmisartan.

INTRODUCTION

Atorvastatin calcium is (βR,dR)-2-(4-fluorophenyl)- β, d- dihydroxy- 5- (1-methyl ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrollo-1-heptanoic acid, calcium salt[1]. Losartan, an angiotensin II receptor antagonist, chemically known as I,2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-imidazole-5-methanol mono potassium salt[2]. It is indicated for the management of hypertension. Extensive literature survey revealed several analytical methods for the estimation of Atorvastatin [3-5] and losartan [6-13] either alone or in combination.

Literature survey reveals few LC methods for the determination of ATV or LTN alone, which indicates the need for method development for the quantitative estimation of ATV in combination with LTN. Hence an attempt has been made to develop and validate a sensitive, reproducible and specific method for the quantitation of ATV and LTN in mixtures.

MATERIALS AND METHODS

Apparatus

The Schimadzu chromatographic system consisted of prominence LC20AD binary pump, rheodyne injector, with 20µl loop, UV/Visible detector and sphinchrom software. LUNA C18, (250mm x 4.6mm id) was used for the separation and quantification of mixtures. An ELICO (LI 610) pH meter was used for the adjustment of pH in the preparation of buffer.

Reagents and Standards

HPLC grade (Methanol and water), AR grade (potassium dihydrogen ortho phosphate and sodium hydroxide) were procured from E.Merck Ltd. Mumbai, India. Atorvastatin and losartan were procured from Aurobindo Pharma Ltd, Hyderabad, India.

Preparation of Buffer
Phosphate buffer pH (6.8) was prepared by adding 22.4ml of 0.1N sodium hydroxide to 50 ml of 0.02M potassium dihydrogen ortho phosphate solution and diluting to 200ml with water. The prepared buffer was then filtered through 0.42µ membrane filter. The mobile phase was degassed using bath sonicator.

PROCEDURE
Preparation of stock solutions, working solutions and calibration standards
Stock solution of ATV, LTN and IS were prepared in methanol at the concentration of 1mg/ml. Working solutions were prepared on the day of analysis by further dilution of the stock solution at the concentration of 0.060-0.120 mg/mL for ATV and 0.080-0.180 for LTN using methanol and spiked with 0.100 µg/ml of IS. All the solutions were filtered using 0.42µm membrane filter and sonicated for 30 min.

Chromatographic conditions
Chromatographic separation was achieved at ambient temperature on C18 column using the mobile phase, methanol: buffer (80:20%, v/v) at the flow rate of 1ml/min and at 238nm.

Pharmaceutical sample preparation
Twenty tablets were weighed and powdered. The quantity of powder equivalent to 10 mg of ATV was taken and 5 ml of methanol was added, sonicated for 10min, filtered and diluted to 10ml with methanol. From the above said solution, 5 ml was diluted to 10 ml with methanol, degassed, filtered. General procedure for HPLC method described under calibration was followed and the concentration of ATV/LTN was estimated.

RESULTS AND DISCUSSION
Method development
The selection of the buffer and the composition of mobile phase were studied and optimized. Separation was found to be satisfactory with methanol and buffer (pH 6.8) in the ratio of 80:20 %, v/v. Increasing the buffer concentration led to very shorter retention time and distortion of the peak. UV detection was carried out at 238nm where both the drugs exhibit maximum absorption. Isocratic mode was chosen as the retention for both the drugs were less than 6 min at a flow rate of 1ml/min. Retention time for ATV and LTN were 4.2 and3.3 min respectively.

METHOD VALIDATION
The developed method was validated for several parameters including linearity, precision, accuracy, sensitivity, robustness and system suitability.

Specificity
Specificity is the ability of the method to measure the analyte response in the presence of interfering substances. This was examined by the validation parameters obtained from the chromatogram. The theoretical plates and tailing factor was found to be 10080 and 0.007 for ATV; 7744 and 0.815 for LTN respectively. The responses were found to be satisfactory which indicates the system suitability of the method.

Linearity
Calibration plot was established by analyzing a series of different concentrations of each compound. In the present study eight concentrations were chosen ranging between 60-120 µg/ml for ATV, and 90-180 µg/ml for LTN. Each concentration was repeated three times.

Accuracy and precision
The accuracy and intraday precision of pure ATV and LTN at three different concentrations were analysed in six replicates. The mean percentage recoveries and their standard deviations for the proposed method for six replicates of ATV and LTN were calculated and found to be satisfactory. Consequently, the interference by the excipients in pharmaceutical formulations was not observed.

Ruggedness
Intermediate precision of six replicate determinations of ATV/LTN was analysed by three analysts on different days. The percentage RSD of assay was found to be less than 2.0%.

Robustness
The chromatographic separation was not influenced by minor variations on pH of the buffer by ±0.2 pH units as well as flow rate ± 0.1 ml/min.

Limit of quantitation and limit of detection
LOD and LOQ were established as per ICH recommendations, based on the approach of S.D of the response and the slope. The detection limits were found to be 0.053 and 0.028 for ATV and LTN respectively. The quantitation limits were found to be 0.162 and 0.085 for ATV and LTN respectively.

Analysis of tablets

The proposed HPLC method was applied to the simultaneous estimation of ATV and LTN in commercial tablets. Satisfactory results were obtained for each compound in good agreement with label claim.

CONCLUSION

The proposed HPLC method is simple, rapid and specific. A clear resolution was achieved even when applied to the formulations. The method is characterized by shorter retention time and without interference from excipients. The statistical measures and recovery studies indicated that the proposed method could be extended for effective quantitation of ATV and LTN in tablet formulation.

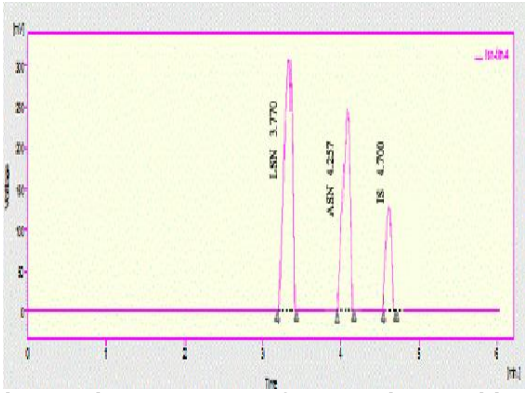

Fig. 1: Chromatogram of LTN and ATV with IS

Table 1: Linearity and regression parameters for ATV/LTN

Drug	Range µg/ml	Regression equation	Correlation coefficient
ATV	200-500	Y=752.57X-2388.57	0.9997
LTN	30-80	Y=5993.14X-2602.79	0.9997

Table 3: Recovery studies and assay results of ATV and LTN

Drug	Added	Measured	Mean % recovery ±S.D	%label claim
ATV	200	202.41	100.58	98.36
	250	249.32		
	300	302.54		
LTN	30	31.21	102.8	101.36
	40	40.9		
	50	52.1		

REFERENCES

1. Indian Pharmacopoeia, Government of India, The controller of publication, New Delhi. 2007;2:749.
2. Sweetman SC and Martindale EI. The Extra Pharmacopoeia, Royal pharmaceutical society. The pharmaceutical press, London. 2002;34:90.
3. Jamshidi A and Nateghi A. HPTLC determination of atorvastatin in plasma, Chromatographia. 2007;65:763-766.
4. Chaudhari BG, Patel NM, Shah PB and Modi KP. Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and Ezetimibe, Indian journal of pharmaceutical science. 2006;68:793 -796.
5. Patil UP, Gandhi SV, Sengar MR and Rajmane VS. Simultaneous estimation

of atorvastatin calcium and telmisartan in tablet dosage form by spectrophotometry, International journal of Chemtech research. 2009;1:970-973.
6. Polinko M, Riffel K, Song H, and Man-wai LO. Simultaneous determination of losartan and EXP 3174 in human plasma and urine utilizing liquid chromatography/ tandom mass spectrometry. J Pharm Biomed Anal. 2003;33:73-84.
7. Williams RC, Alasandro MS, Fasone VL, Boucher RJ and Edwards JF. Comparison of liquid chromatograghy, Capillary electrophoresis and Supercritical fluid chromatography in the determination of losartan potassium drug substance in Cozaar tablets. J Pharm Biomed Anal. 1996;14:1539-1546.
8. McCarthy KE, Qingxi Wang tsai EW, Gilbert RE, Ip DP and Brooks MA. Determination of losartan and its degradates in COZZAR tablets by reversed- phase high performance thin layer chromatography. J Pharm Biomed Anal. 17:671-677.
9. Maurer HH, Kraemer JW and Arlt. Screening for the detection of angiostensin converting enzyme inhibitors,their metabolites and AT II receptor antagonists, their drug monitoring. 1998;20:706-713.
10. Furtek CI and Lo MW. Simultaneous determination of a novel angiostensin II receptor blocking agent, Losartan and its metabolite in human plasma and urine by high performance liquid chromatography. J Chroma Biomed Appn. 1992;573:295-301.
11. Lee H, Shim HO and Lee HS. Simultaneous determination of Losartan and active metabolite EXP3174 in rat plasma by HPLC column switching. Chromatographia. 1996; 42:39-42.
12. Walily AF, Belal SF, and Heaba EA. An improved method for the simultaneous determination of Losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Pharma Biomed Anal. 1997;15:1021-1029.
13. Farthing D, Sica D, Fakhry I, Pedro A and Gehr TWB. Simple high-performance chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate. J Chromatogr B. 1997;704:374-378.