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RESEARCH ARTICLE

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALISKIREN AND VALSARTAN IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise and accurate RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Aliskiren (ALN) and Valsartan (VLN) from their combination dosage form. Water's HPLC equipped with UV-Visible with Empower -2 software was used. Chromatographic separation was achieved isocratically on a Hiber@ Lichrosphere ® C18 column (250×4.6 mm, 5 µ particle size) using a mobile phase, Methanol and Potassium Di Hydrogen Phosphate buffer and Acetonitrile (adjusted to pH 3.0 with 1% orthophosphoric acid) in the ratio of 50:30:20v/v/v. and UV detection was carried out at 271 nm for ALN and VAN, respectively. The retention time for Aliskiren and Valsartan was found to be 6.92 and 7.91 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form. The method was found linear over the range of 10-50µg /ml for Aliskiren and 10-50 µg / ml for Valsartan. The proposed method was validated as per the ICH and USP guidelines.

Key words: RP-HPLC Method**;** Aliskiren hemifumarate; Valsartan; Tablet dosage forms.

INTRODUCTION

Aliskiren,(2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4 hydroxy2,7 diisopropyl -8-[4-methoxy-3-(3-methoxypropoxy)phenyl] octanamide hemifumarate)[1-2] (Fig.1). The first oral direct renin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II.

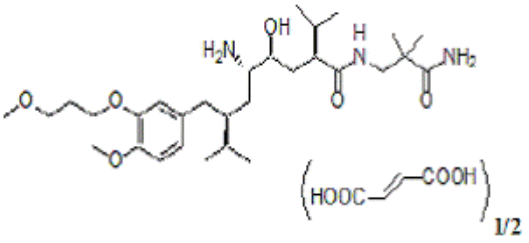

Figure 1; Chemical structure of Aliskiren

Valsartan (VAL) is chemically, N - (1 - oxopentyl) - N - [(2' - (1H - tetrazol - 5 - yl) (1, 1' - biphenyl) - 4 - yl) methyl] - L – valine (Fig. 2), is a potent angiotensin receptor blocker.

Literature survey reveals the availability of several methods for estimation of both Aliskiren and Valsartan [3] and Valsartan [4]includes UV, HPLC as alone or in combination with other drugs. No method has been reported for the estimation of Aliskiren and Valsartan in combined dosage form. Present work emphasizes on the quantitative estimation of Aliskiren and Valsartan in their combined dosage form (Valturna) by RP-HPLC.

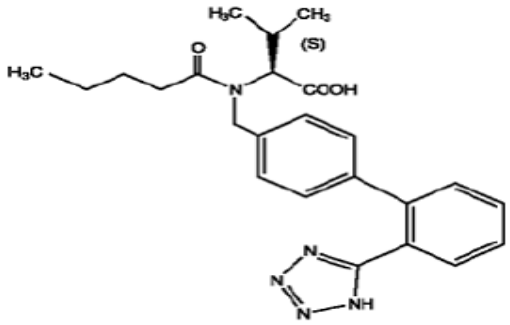

Figure 2: Chemical structure of Valsartan

MATERIALS AND METHODS

Experimental

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Hiber@ Lichrosphere ® C_{18} column (250mmx4.6mm; 5µm), a 515 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software. The UV spectrum of the drugs was taken using a shimadz-1800 UV-Visible spectrophotometer.

Instrumentation

The present work was carried out on Water's HPLC equipped with UV-Visible detectors with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade

and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Chemicals and Solvents

The Aliskiren and Valsartan Working standards were kindly gifted by Dr .reddys Laboratories Hyderabad. And *Morepen Laboratories Ltd, New Delhi*-India. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer (pH 3.0)

Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

500 ml of the methanol and phosphate buffer 300 ml was mixed with 200 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum.

Procedure

A mixture of methanol and buffer and acetonitrile in the ratio of 50:30:20 v/v was found to be the most suitable mobile phase for ideal separation of Aliskiren and Valsartan. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 1700 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 271 nm. The run time was set at 10 min. Under these optimized chromatographic conditions the retention time obtained for the drugs Aliskiren and Valsartan were 6.92 min and 7.9 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

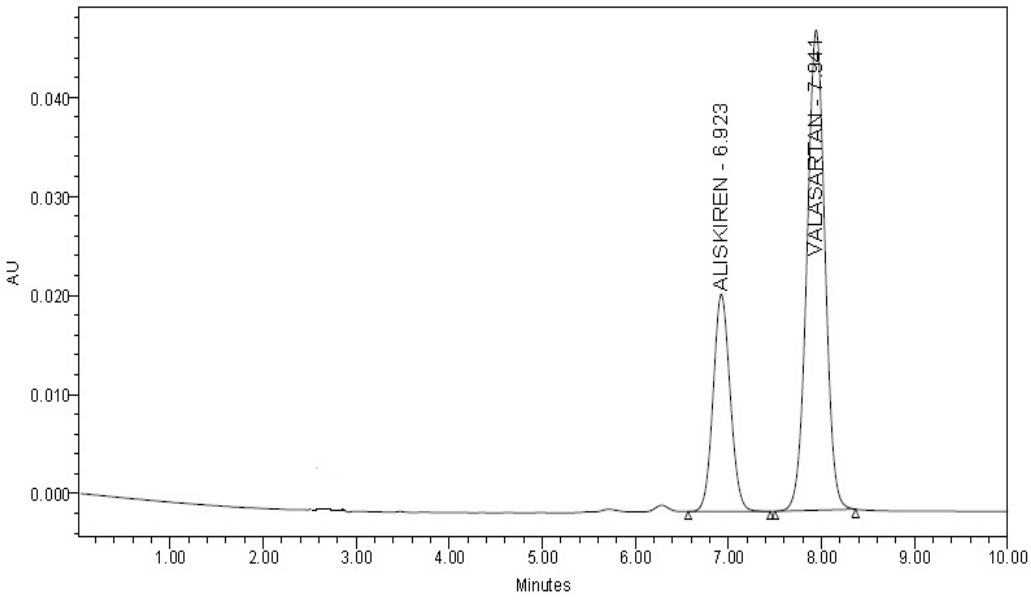

Figure 3: Typical chromatogram for Aliskiren and Valsartan.

Calibration plot

About 100 mg of Aliskiren and 100 mg of valsartan was weighed accurately,transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:30:20 v/v mixture of methanol and Phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000µg/ml solution. From this, a working standard solution of the drugs (20µg/ml for Aliskiren and 20µg/ml for Valsartan) was prepared by diluting the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10-50µg/ml for Aliskiren and 10-50µg/ml for Valsartan) were prepared from the solution in10 ml volumetric flasks using the above diluents.20 µl of each dilution was injected six times into the column at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-50µg/ml for Aliskiren and 10-50µg/ml for Valsartan. The relevant data are furnished in Table 1&2 and fig.4&5. The regression equations of this curves was computed. This regression equation was later used to estimate the amount of Aliskiren and valsartan in tablets dosage forms.

Table 1: Calibration data for Aliskiren

S.No	Concentration (µg/ml)	Peak Area (n=5)
1.	10	278636
2.	20	498881
3.	30	784446
4.	40	1042985
5.	50	1283891

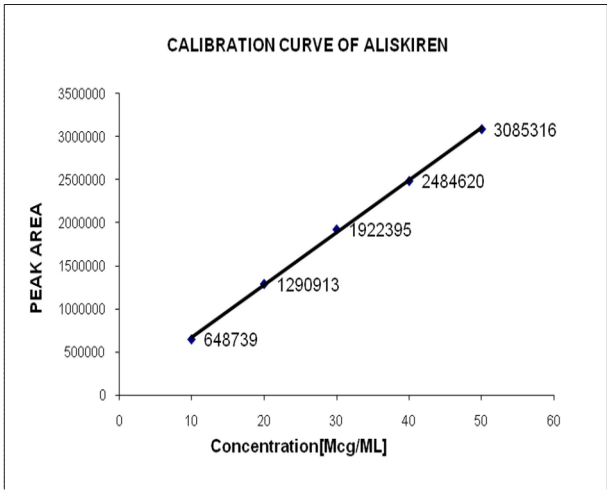

Figure 4: Calibration graph for Aliskiren.

Table 2: Calibration data for Valsartan

S.No	Concentration (µg/ml)	Peak Area (n=5)
1.	10	648739
2.	20	1290913
3.	30	1922395
4.	40	2484620
5.	50	3085316

Validation of the proposed method[5-9]

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of Aliskiren and valsartan.

Solution containing 20µg/ml for Aliskiren and 20µg/ml for valsartan was subjected to the proposed HPLC analysis to check intra-day and interday variation of the method and the results are furnished in Table 3&4. The accuracy of the HPLC method was assessed by analyzing solutions of Aliskiren and valsartan at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table 5. The system suitability parameters are given in Table 6.

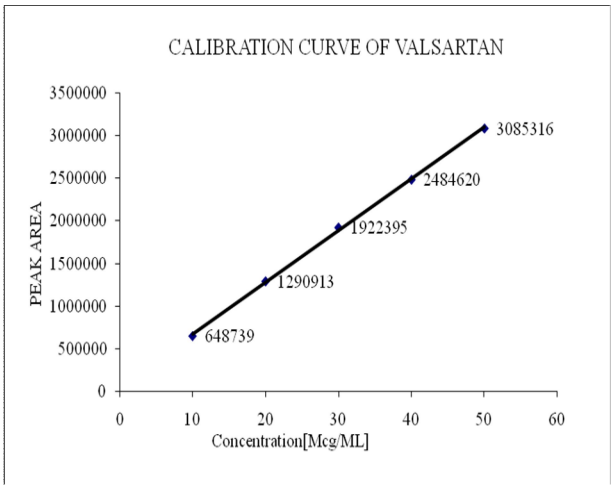

Figure 5: Calibration graph for Valsartan

Table 3 .Precision of the proposed HPLC method (Aliskiren)

S.NO.	Concentration of Aliskiren (20µg/ml)	Peak area	
		Intra-day	Inter-day
1.	Injection-1	456881	498581
2.	Injection-2	478581	498781
3.	Injection-3	497861	498681
4.	Injection-4	498581	498781
5.	Injection-5	497781	498781
6.	**Average**	485937	498721
7.	**Standard Deviation**	18307.75	89.44272
8.	**%RSD**	3.767515	0.017934

Table 4 .Precision of the proposed HPLC method (Valsartan)

S.NO.	Concentration of valsartan(20µg/ml)	Peak area	
		Intra-day	Inter-day
1.	Injection-1	1289943	1289943
2.	Injection-2	1290953	1290953
3.	Injection-3	1299913	1299913
4.	Injection-4	1298913	1298913
5.	Injection-5	1297913	1297913
6.	**Average**	1295527	1295527
7.	**Standard Deviation**	4703.656	4703.656
8.	**%RSD**	0.363069	0.363069

 ISSN: 2250-1177 CODEN: JDDTAO

Table 5: Accuracy studies

S.No	Concentration	Amount added (mg)		Amount found (mg)		% Recovery		% Mean recovery
		ALN	VLN	ALN	VLN	ALN	VLN	
1	50%	5.0	5.03	5.03	5.15	98.40	97.66	98.03
2	100%	10.1	10.04	9.97	10.03	100.59	99.40	99.95
3	150%	15.0	15.02	14.96	15.1	99.86	99.07	99.20

Estimation of Aliskiren and valsartan in tablet dosage forms.

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate Aliskiren and valsartan in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100 mg of Aliskiren and and 100 mg of valsartan was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:30:20 v/v mixture of methanol and phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45µ membrane filter. This solution was further diluted to get the required concentrations. This solution was injected into the column six times. The average peak area of the drugs was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 7.

Table 6: System suitability parameters

Parameter	Result (Aliskiren)	Result (Valsartan)
Linearity (µg/ml)	10-50	10-50
Correlation coefficient	0.999	0.999
Tailing factor	1.25	1.57
LOD (µg/ml)	1.20	0.25
LOQ(µg/ml)	3.6	0.42

Table 7: Assay Results for Tablets Using the Proposed Method

Label claim (Valturna-150/160mg)		Amount found (mg)		% Amount found	
ALN	VLN	ALN	VLN	ALN	VLN
150	160	150.03	159.75	99.98	99.84

RESULTS AND DISCUSSION

In the proposed method, the retention time of Aliskiren and valsartan was found to be 6.9 min and 7.9 min. Quantification was linear in the concentration range of 10-50µg/ml for Aliskiren and 10-50µg/ml for valsartan. The regression equation of the linearity plot of concentration of Aliskiren over its peak area was found to be $Y=25546.14+11383.6X$ ($r^2=0.999$) for Aliskiren and $Y=60668.61+66338.37X$ ($r^2=0.999$) for valsartan, where X is the concentration of Aliskiren and valsartan (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 6083 for Aliskiren and 3510 for valsartan, which indicates efficient performance of the column. The limit of detection and limit of quantification for Aliskiren were found to be 0.02 µg/ml and 0.08 µg/ml and for valsartan were found to be 0.01 µg/ml and 0.03 µg/ml respectively, which indicate the sensitivity of the method. The use of methanol and phosphate buffer and acetonitrile in the ratio of 50:30: 20 v/v resulted in peak with good shape and resolution.

The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method

CONCLUSION

The developed UV spectroscopic method was validated and the statistical validation was performed with the simplicity and ease of operation ensures that the validated method can successfully used for routine analysis of Aliskiren hemifumarate and Valsartan in bulk and tablet dosage formulation.

ACKNOWLEDGEMENTS:

The authors would like to thanks to Dr.Reddy's Laboratories, Hyderabad,and Morpean Laboratories ,New Delhi for providing the gift samples of Aliskiren/ Valsartan for the project work. The authors are thankful to D. Manohar Reddy, Chairman of Trinity College of Pharmaceutical Sciences for their kind help and providing all necessary facilities.

DECLARATION OF INTEREST:

The authors have no conflicts of interest

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 ISSN: 2250-1177 CODEN: JDDTAO