Simultaneous Development and Validation of Valsartan and Sacubitril by RP-HPLCMethod in Tablet Formulation

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Abstract

For Valsartan and Sacubitril in combined tablet dose form, a novel, sensitive, accurate, and reliable reversed-phase high performance liquid chromatographic testing method was designed and validated. Chromatographic separation was carried out using a gradient HPLC system on a C18 column (Kromasil ODS, 250 x 4.6 mm, 5) with a mobile phase of buffer and acetonitrile in a ratio of 55:45% v/v/v and UV detection at 254nm. The retention time of Sacubitril and Valsartan was found out to be 9.0 and 5.2 min. This RP-HPLC method was validated as per ICH guidelines for parameters like specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and system suitability. The validation results obtained were within specified limits.

Keywords: *Valsartan, Sacubitril, RP-HPLC, quantification and linearity.*

1. Introduction

Valsartan [1, 2], chemically N-(1-oxopentyl)-N-[[2'-(1Htetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl] - L-valine [Figure 1] is an Angiotensin II receptor blocker, which is used to treat hypertension, diabetic nephropathy and heart failure. Sacubitril is chemically 4-[[(2S,4R)- 5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl) pentan-2-yl] amino]-4-oxobutanoic acid, which produces prodrug neprilysin inhibitor, that is used in combination with Valsartan to reduce the risk of cardiovascular ailments in patients reported with chronic cardiac failure. Combination of these two drugs is available with a brand name of Azmarda-50, containing 26mg and 24mg label claims of Valsartan and Sacubitril is used in the treatment and prevention for chronic cardiac failure and other cardiac related conditions.

In accordance with the literature, analytical techniques for visible UV, TLC, and a few HPLC are available. The current work is aimed at developing a simple, rapid, exact, and reliable RP-HPLC method for the simultaneous separation, identification, and determination of Valsartan and Sacubitril in pharmaceutical formulations and bulk. The suggested procedure was approved in accordance with ICH rules.

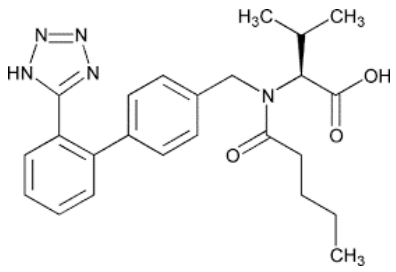

Figure 1. Structure of Valsartan

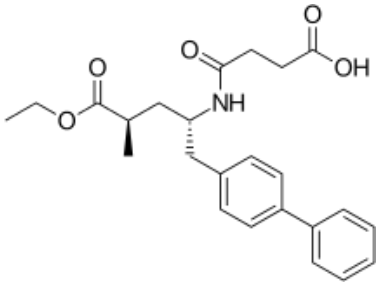

Figure 2. Structure of Sacubitril

2. Materials and methods

2.1 Instruments: In this study the present was carried on HPLC **(**WATER**S)**, which comes with an auto sampler injector with variable UV detector and with a software of Empower-2.

2.2 Reagents & Chemicals: Valsartan and Sacubitril were obtained as gifted samples. Methanol, Acetonitrile is of HPLC grade and MilliQ water was used. For the assay, Azmarda-50, which contains 26mg and 24mg label claim of Valsartan and Sacubitril were procured from the local market.

2.3 Mobile phase preparation: Prepare, filter and degass the mixture of buffer and Acetonitrile in the ratio of 55; 45 %v/v/v.

2.4 Buffer preparation: Weigh and dissolve about the 2.72mg of potassium dihydrogen phosphate in to 1000ml of water and mixed will adjust the pH to 3.5 with diluted orthophosphoric acid solution filter through Millipore PVDF membrane filter.

2.5 Diluent Preparation: Prepare the required volume of degassed mixture of methanol and water in the ratio of 80:20 v/v.

2.6 Preparation of solutions

2.6.1 Standard solution: By transferring and combining 100 mg each of the 99.7% pure standard samples for Valsartan and Sacubitril into 100 mL volumetric flasks, the primary stock solution with 1000 g/mL of each was done separately. From these five working standard solutions of concentrations covering the range of 50-150µg/mL for Valsartan and Sacubitril were prepared by transferring and diluting different aliquots into a series of 10mL volumetric flasks with the same diluent.

2.6.2 Sample solution: Weighed and transferred 10 tablets of AZMARDA 50 into a mortar and pestle. Crush these tablets into fine powder. And add 20 ml of milliQ water. Stir on a magnetic stirrer using appropriate magnetic bead until the tablet is completely disperse. After removing the bead by washing with about 10 ml of methanol. Add 40 ml of methanol and sonicated at room temperature for about 45 minutes with every 5 minutes intermittent shaking. Finally dilute the volume about 1cm below the mark with methanol and mix well. Allow the solution to equilibrate to room temperature and dilute to volume with methanol and mix well. Centrifuge a portion of the solution at 5000 rpm for about 10 minutes. Further transfer 4 ml of clear supernatant liquid in to 100 ml volumetric flask dilute to volume, filter a portion of the solution by using 0.45 micro membrane PVDF filter.

3. Results and Discussion

3.1 Method Development

3.1.1 Optimized Chromatographic method: In the current analysis the separation of Valsartan and Sacubitril was done by C18 column (Kromasil,ODS 5µ, 250 mm × 4.6 mm) with mobile phase of buffer (pH-3.5) and Acetonitrile in the ratio of 55:45 %v/v/v at a flow rate was 1.3 mL/min with UV detection wavelength of 254nm. The retention times for Valsartan and Sacubitril were found to be 5.2 and 9.0 min.

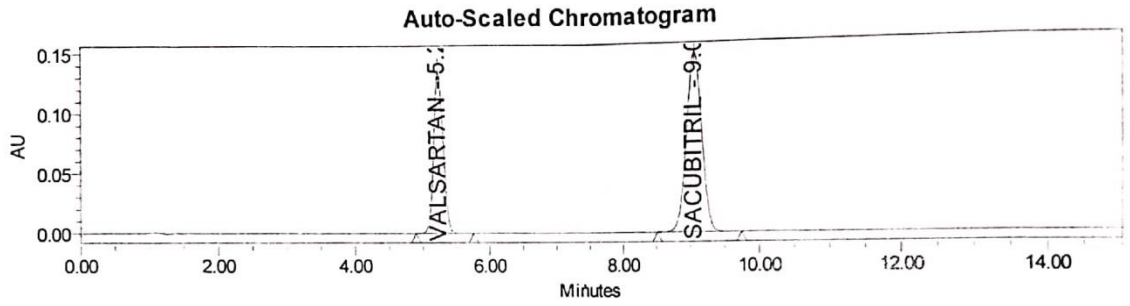

Figure 3. Chromatogram of Valsartan and Sacubitril

3.2 Method Validation

3.2.1 Specificity: Specificity was tested against standard compounds and interferences in the peaks of blank and placebo under optimized chromatographic conditions. The comparison of the chromatograms of blank and placebo mixture reveals that there were no additional peaks of Valsartan and Sacubitril in the sample solution.

3.2.2 System suitability: System suitability parameters like number of theoretical plates, HETP and peak tailingwere determined for Valsartan and Sacubitril were determined.

Table 1. System suitability data of Valsartan and Sacubitril

Parameter	Valsartan	Sacubitril
Retention time	9.0	5.2
Theoretical plates	6586	7735
Tailing factor	1.03	1.01
% RSD	0.18	0.16

3.2.3 Linearity: The linearity of the proposed method was conducted by analyzing working standard solutions of Valsartan and Sacubitril of five different concentrations. 20µl of each solution of different concentrations were injected into the HPLC system. The peak areas of the chromatograms obtained for each concentration of the drug solution were noted and recorded. Plotting the determined peak area ratio vs the applied concentrations of Valsartan and Sacubitril resulted in separate calibration curves for each drug. The linearity of the calibration graphs were validated by the high values of correlation coefficients 0.9999 and 0.9999with slope and intercept values of 10468.8 and 13217.4 for Valsartan and 21550.4 and 17492.6 for Sacubitril respectively (Table 2).The LOD of Valsartan and Sacubitril were found to be 0.036µg mL^{-1} and 0.034519µg mL^{-1}, respectively and the LOQ values of Valsartan and Sacubitril were 0.121µg mL^{-1} and 0.114µg mL^{-1}.

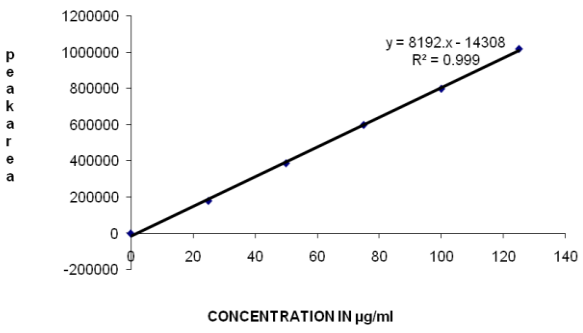

Figure 4. Calibration curve of Valsartan

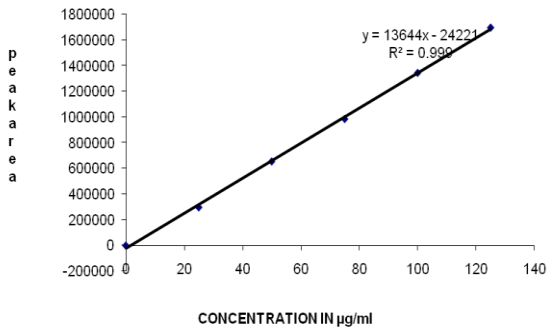

Figure 4. Calibration curve of Sacubitril

Table 2. Results of linearity studies of Valsartan and Sacubitril

Concentration (µg/ml)	Peak area of Valsartan	Peak area of Sacubitril
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

3.2.4 Precision: The precision studies were carried out by replicate injections (Intra and inter-day precision studies) of tablet powder. The intra and inter-day precision studies for five sample preparations showed a %RSD of 0.06 % for Valsartan and 0.08 %.

Table 3. Precision data for Valsartan

S.No	RT	Area
Injection1	5.2	1324732
Injection2	5.252	1324724
Injection3	5.243	1324305
Injection4	5.246	1323106
Injection5	5.254	1325156
*Mean		1324405
*Std. Dev.		785.8
*%RSD		0.06

Table 4. Precision data for Sacubitril

S.No	RT	Area
Injection1	9.066	2306222
Injection 2	9.067	2305299
Injection 3	9.059	2303464
Injection 4	9.063	2303450
Injection 5	9.068	2307525
*Mean		2305192
*Std. Dev.		1770.3
*%RSD		0.08

3.2.5 Accuracy: The accuracy was performed at three levels, with concentrations of 50, 100 and 150% of labeled amount of Valsartan and Sacubitril. Three replicate samples of each concentration level were prepared and the % recovery at each level was determined.

Table 5. Accuracy data for Valsartan and Accuracy data for Sacubitril

Sample Id	Conc found (µg/ml)	Conc. Obtained(µg/ml)		% Recovery		Mean recovery		Statistical Analysis %RSD	
		VAL	SAC	VAL	SAC	VAL	SAC	VAL	SAC
50%	5	5.01	4.92	100.2	98.0	**99.73**	**99.2**	**0.505**	**1.2**
50%	5	4.96	4.96	99.2	99.2				
50%	5	4.99	5.02	99.8	100.4				
100%	10	9.95	9.95	99.5	99.5	**98.8**	**99.5**	**0.66**	**0.2**
100%	10	9.87	9.94	98.7	99.4				
100%	10	9.82	9.98	98.2	99.8				
150%	15	14.64	14.78	97.6	98.6	**98.8**	**99.0**	**1.45**	**0.530**
150%	15	14.76	14.94	98.4	99.6				
150%	15	15.06	14.83	100.4	98.8				

3.2.6 Robustness studies: The robustness is deliberate changes to the parameters like mobile phase ratio, pH of the solution and detection wavelength. The factors evaluated in the study were the change in flow rate by ±0.2mL min^{-1} and the change in detection wavelength by ±2nm. The developed method was found to be robust enough that the peak areas of Valsartan and Sacubitril were not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits.

Table 7: Results of robustness studies of Valsartan and Sacubitril

Std. Replicate	Variation in flow rate				Variation in Mobile phase composition			
	Flow Rate 0.8ml/min		Flow Rate 1.2ml/min		Buffer: Methanol (40:60)		Buffer: Methanol (30:70)	
	VAL	SAC	VAL	SAC	VAL	SAC	VAL	SAC
1	2492492	1500192	1676589	100524	1951632	1196996	1979168	1153397
2	2495874	1500426	1675428	100468	1954783	1198547	1967452	1154782
Mean	2494183	1500309	1676009	100496	1953208.0	1197772	1973310	1154090
SD	2391.4	165.5	820.9	39.59	2228.0	1096.2	8284.46	979.34

%RSD	0.09	0.01	0.04	0.03	0.11	0.09	0.4	0.08
RT	3.150	4.674	2.168	3.121	2.618	4.394	2.572	3.331
TF	1.4	1.2	1.3	1.2	1.3	1.2	1.3	1.2
TP	5752	7187	4207	5412	4577	6498	4476	6471

3.2.7 Ruggedness: The ruggedness of this method was evaluated by a different analysts and different instruments in the same laboratory. The % RSD for peak areas of Valsartan and Sacubitril was calculated, these results revealed that the %RSD was within the limits (<2.0) indicating that the developed method was found to be rugged.

3.2.8 Analysis of marketed formulation: Analysis of marketed tablets [Azmarda-50] was carried out using the same mobile phase and foresaid mentioned conditions. The % content of Valsartan and Sacubitril in Azmarda-50 tablets were calculated and found to be 99.88 and 104.21 %, thus the assay of Valsartan and Sacubitril in tablet dosage forms was accurate and within the acceptance level.

Table 8: Assay results of Valsartan and Sacubitril in tablet formulations

Drug Name	uantity Label Claim(mg)	Area of the sample	% Assay	*%LA
Valsartan	26	2354393	104.2	104.2
Sacubitril	24	2352954	104.2	104.2

4. Conclusion

The proposed newly developed method is selective, accurate and sensitive method for the simultaneous estimation of Valsartan and Sacubitril in pure and marketed formulations with good resolution, shorter resolution time and with lesser consumption of analytical reagents. The validation results indicate that the proposed RP-HPLC method is suitable for the routine quality control of Valsartan and Sacubitril in combined dosage forms and required short time for analysis and can be approved for testing laboratories.

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