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Assay of Valsartan and Sacubitril in Combined Dosage Form by RP-HPLC (Method Development and Validation)

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ABSTRACT

A new, sensitive, precise, and accurate reversed-phase-high performance liquid chromatographic assay method for Valsartan and Sacubitril in combined tablet dosage form was developed and validated. Chromatographic separation was optimized by gradient HPLC on a C18 column [Inertsil ODS, 250 x 4.6 mm, 5µ] utilizing a mobile phase consisting of mixture of buffer (pH-2.7), acetonitrile and methanol in the ratio of 25:60:15 % v/v/v in the ratio of 30:50:20 %v/v at a flow rate of 1.0mL/min with UV detection at 245nm. The retention time of sacubitril and valsartan was 3.407 and 4.280 min respectively. The developed reversed-phase-high performance liquid chromatographic (RP-HPLC) method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and system suitability. Good linearity obtained over the range of 50µg mL^{-1} to 150µg mL^{-1} for valsartan and sacubitril. The correlation coefficient was found to be 0.9987 and 0.9988 for valsartan and sacubitril respectively. The % RSD of precision was found to be 0.0478 and 0.158% for valsartan and 0.0355 and 0.154% for sacubitril respectively. The % mean recovery was found to be 99.92 to 99.84 % for valsartan and 101.14 % to 101.28 % for sacubitril respectively. The results obtained for accuracy, precision, LOD, LOQ and ruggedness were within limits.

Keywords: Valsartan, Sacubitril, Reversed-phase-high performance liquid chromatographic method.

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-receptor blocker (ARB) is used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Sacubitril [3, 4] is chemically 4-[[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl) pentan-2-yl] amino]-4-oxobutanoic acid [Figure 2], prodrug neprilysin inhibitor is used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure and reduced ejection fraction. Combination of these two drugs is available in local pharmacy in the brand name Azmarda-50 manufactured by Cipla labs containing 26mg and 24mg label claims of valsartan and sacubitril is used for the treatment and prevention drug for chronic heart failure and other heart conditions.

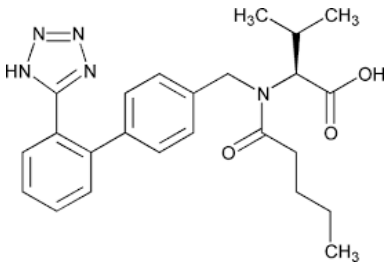

Figure 1: Molecualr structure of Valsartan

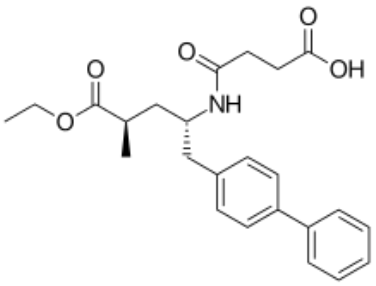

Figure 2: Molecualr structure of Sacubitril

Till date, only six RP-HPLC methods [5-10] were reported in the literature for the determination of valsartan and sacubitril in combined dosage forms resulted in high base noise, long tailing, long retention times and low sensitivity. Hence, an attempt was made to develop a new method for simultaneous estimation and validation of valsartan and sacubitril in combined dosage forms employing RP-HPLC technique in accordance with the International Conference on Harmonization (ICH) guidelines and was succeeded in developing the method.

MATERIALS AND METHODS

Instrumentation: The present separation valsartan and sacubitril was carried on a PEAK chromatographic system [WATERS HPLC, Model: 2695] equipped with an automated sample injector of 20µl fixed volume loop with, variable wavelength programmable UV detector [UV-2487].The output signal was monitored and integrated by Empower 2 software. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis. Sonicator (1.5L) Ultrasonicator (SE-60US) was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using electronic analytical balance (SAB-224CL) and pH of the mobile phase was adjusted by using digital pH meter (PH-7000) respectively.

Chemicals used: Original samples of valsartan and sacubitril (99.7% pure) were obtained as gifted samples from Cipla Labs, India. Methnol (HPLC Grade), Acetonitrile (HPLC grade) and Trifluroacetic acid and Formic acid (AR grade) were purchased from Merck India and were used as obtained. All the dilutions were performed in standard class-A, volumetric glassware. For the assay of commercial formulation, Azmarda-50 manufactured by Cipla Labs containing 26mg and 24mg label claims of valsartan and sacubitril were procured from the local market. Milli Q water was used throughout the analysis.

Mobile phase preparation: Prepare a filtered and degassed mixture of buffer (pH-2.7) and Acetonitrile and methanol in the ratio of 25:60:15 %v/v/v respectively.

Buffer preparation (pH-2.7): Prepared by dissolving 1.0mL of 88 - 100 % formic acid to a 1000 mL graduated cylinder using a volumetric pipette and dilute to a final volume of 1000mL with deionized water and mix well.

Diluent Preparation: In the present assay water and acetonitrile in the ratio of 40:60%v/v was used as diluent.

Preparation of solutions

Standard solution: Primary stock solution containing 1000µg/mL of valsartan and sacubitril were prepared separately by transferring and dissolving 100mg of valsartan and sacubitril standard powder (99.7% pure) separately into 100mL of volumetric flasks by dilute with the diluents. From this 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.

Sample solution: Weighed and transferred 20 tablets of AZMARDA 50 [Containing 26mg and 24mg label claims of valsartan and sacubitril] into a mortar and pestle. Crush the above tablets to fine powder. Weigh and transfer sample powder quantity equivalent to 100mg of valsartan and sacubitril into a 100mL volumetric flask containing 50mL of mobile phase and shaken vigorously, sonicated for 15min and volume made up to the mark with mobile phase. Aliquots of the above solution was pipetted and transferred into a series of cleaned, dry 10mL volumetric flasks and the diluent was added up to the mark to get final concentration of 50-150µg/mL for valsartan and sacubitril respectively. 20µL volume each of these standard and sample solutions were injected five times and the peak areas were recorded.

RESULTS AND DISCUSSION

Method development: For developing the present HPLC method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant that include the selecting the appropriate wavelength and choice of stationary and mobile phases.

Chromatographic conditions: In the present study the separation of valsartan and sacubitril was achieved by using C18 column (Inertsil,ODS 5µ, 250 mm × 4.6 mm) with mobile phase consisting of mixture of buffer (pH-2.7),Acetonitrile and methanol in the ratio of 25:60:15 %v/v/v at a flow rate was 1.0 mL/min with UV detection wavelength of 245nm at ambient temperature. The retention time for valsartan and sacubitril were found to be 3.407 and 4.280min respectively (Figure 3).

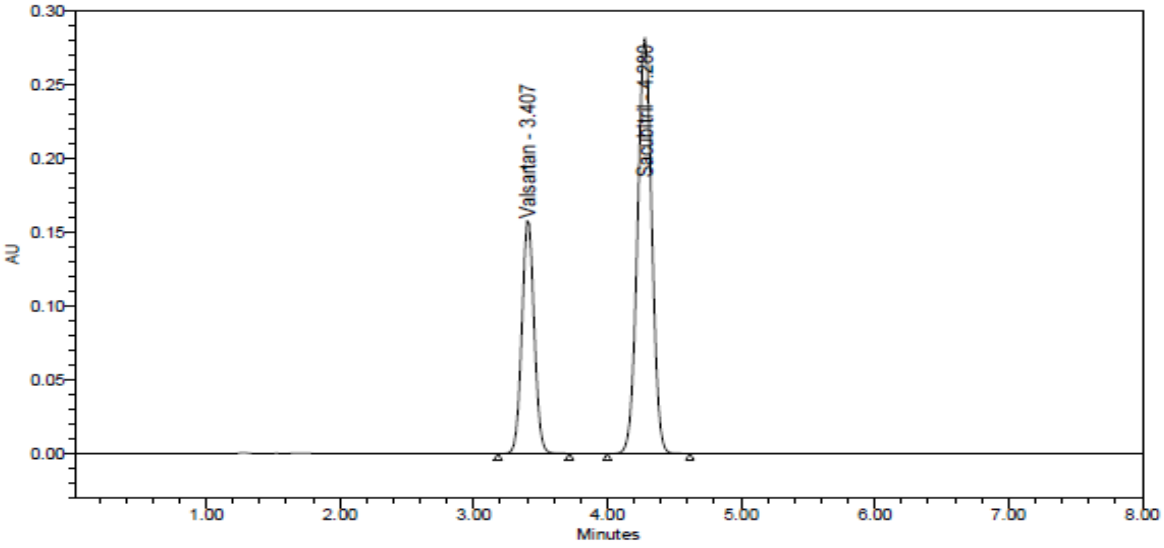

Figure 3. Validated chromatogram of valsartan and sacubitril

Method validation: The developed RP-HPLC method is further validated in accordance with ICH guidelines [11] for assay of valsartan and sacubitril using the following parameters.

Specificity: The specificity of the proposed method was tested against standard compounds and possible interference peaks in the presence of blank and placebo under optimized test conditions. The comparison of the chromatograms of blank and placebo mixture revealed that there were no additional peaks co-eluting with the peaks of valsartan and sacubitril in the sample solution. Moreover no interference from the blank and placebo was observed at the retention time of the valsartan and sacubitril respectively concluding that the proposed method is specific.

System suitability: System suitability parameters like number of theoretical plates, HETP and peak tailing were determined for valsartan and sacubitril by using the above chromatographic conditions and the values for the parameters were reported in **table 3**.

Table 1: System suitability data of Valsartan and Sacubitril

Parameter	Valsartan	Sacubitril
Retention time	3.407	4.280
Theoretical plates	6476	7449
Tailing factor	1.04	1.00
% RSD	0.17	0.15

Linearity and Detector response: The linearity of the proposed method was evaluated by analyzing working standard solutions of valsartan and sacubitril of five different concentrations. Twenty microlitre of each solution of the above said concentrations were injected into the prescribed chromatographic system under operating chromatographic conditions described above. The chromatograms obtained for each concentration of the drug solution were recorded and the peak areas were determined. Separate calibration curves of valsartan and sacubitril were obtained by plotting the peak area ratio determined versus the applied concentrations of valsartan and sacubitril [Figure 4, 5]. The linearity of the calibration graphs were validated by the high values of correlation coefficients 0.9987 and 0.9988 with 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} and are reported in Table 2 respectively.

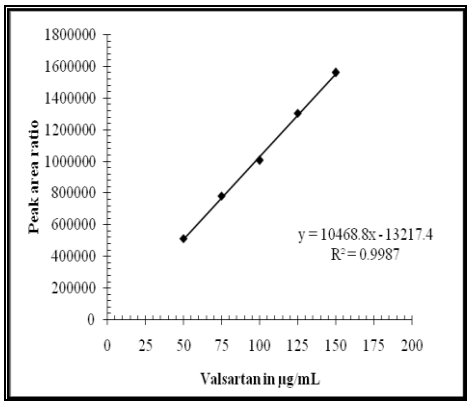

Figure 4: Calibration curve of valsartan

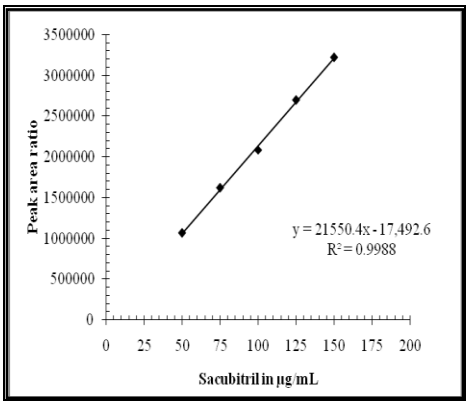

Figure 4: Calibration curve of sacubitril

Table 2: Results of linearity studies of valsartan and sacubitril by the proposed method

S.No	Concentration in 121µg mL^{-1} for valsartan	Peak Area	Concentration in µg mL^{-1} for sacubitril	Peak Area
1	50	512982	50	1064126
2	75	782386	75	1619870
3	100	1007471	100	2087486
4	125	1305404	125	2696833
5	150	1560074	150	3219452
Slope : 10468.8 **Intercept** : 13217.4 **Cc** : 0.9987 **LOD** :0.0364µg/ml **LOQ** :0.121µg/ml			**Slope** : 21550.4 **Intercept** :17492.69690 **Cc** : 0.9988 **LOD** :0.0342µg/ml **LOQ** : 0.114µg/ml	

Precision: The precision studies of the proposed method were ascertained by replicate analysis (Intra and inter-day precision studies) of tablet powder and the results were tabulated in Table 3, 4. The Intra and inter-day precision studies for six sample preparations showed a %RSD of 0.0478 & 0.158% for valsartan and 0.035 & 0.154% for sacubitril respectively revealing the high precision of the proposed RP-HPLC method (Table 3,4**)**.

Table 3: Precision data for Valsartan

S.No	RT	Area	%Assay
Injection1	3.413	1011566	100.1
Injection2	3.411	1011796	99.9
Injection3	3.414	1007737	99.1
Injection4	3.415	1009020	99.7
Injection5	3.411	1009751	99.3
Injection6	3.412	1008849	98.7
*Mean	3.412	1009787	99.5
*Std. Dev.	0.00163	1604.547	0.54
*%RSD	0.0478	0.158	0.54

Table 4: Precision data for Sacubitril

S.No	RT	Area	%Assay
Injection1	4.289	2096084	100.3
Injection 2	4.286	2094997	100.0
Injection 3	4.289	2087417	99.5
Injection 4	4.290	2089975	99.4
Injection 5	4.287	2092058	99.7
Injection 6	4.289	2090676	100.4
*Mean	4.288	2091868	99.9

*Std. Dev.	0.001506	3237.811	0.43
*%RSD	0.035	0.154	0.43

*Average of six determinations

Accuracy: The accuracy of the present proposed method was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 50, 100 and 150% of labeled amount of valsartan and sacubitril in tablets. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3), was determined and reported in (Tables 5, 6**)**. The % of recovery was ranged from 98.20% to 100.7% for valsartan and 98.50% to 100.7% for sacubitril respectively.

Table 5: Accuracy data for Valsartan

S.No	Accuracy Level	Injection	Sample area
1	50%	1	99.1
		2	100.4
		3	99.0
2	100%	1	100.1
		2	99.9
		3	99.1
		4	99.7
		5	99.3
		6	98.7
3	150%	1	100.7
		2	100.5
		3	98.2

Table 6: Accuracy data for Sacubitril

S.No	Accuracy Level	Injection	Sample Area
1	50%	1	100.6
		2	10.3
		3	98.5
2	100%	1	100.3
		2	100.0
		3	99.5
		4	99.4
		5	99.7
		6	100.4
3	150%	1	100.7
		2	100.1
		3	98.8

*Average of Three determinations

Robustness studies: The robustness of the proposed method was verified by making deliberate changes to some parameters such as the mobile phase volume ratio, pH of the solution and detection wavelength. The factors selected in the present 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 and shown in Table 7 respectively.

Table 7: Results of robustness studies of valsartan and sacubitril

Condition	Mean area for valsartan	% Difference	Mean area for sacubitril	% Difference
WL Changes-1 -243nm	1034575	-0.21	2085432	-0.51
WL Changes- 2 -247nm	1017589	0.50	2087732	-0.67
Flow rate changes-1 (0.8)	1000456	-0.32	2088327	-0.89
Flow rate changes-2 (1.2)	1012350	0.24	2099651	-0.98

Ruggedness: The ruggedness of the proposed RP-HPLC method was evaluated by a different analyst and different instrument in the same laboratory. The % RSD for peak areas of valsartan and sacubitril was calculated and the experimental results are shown in Table 8 and these results revealed that the %RSD was within the limits (<2.0) indicating that the developed RP-HPLC method was found to be rugged.

Analysis of marketed formulation: Analysis of marketed tablets [Azmarda-50] was carried out using the above said optimized mobile phase and HPLC conditions. The % content of valsartan and sacubitril in Azmarda-50 tablets were calculated and found to be 99.88 and 101.21 % respectively making the assay of valsartan and sacubitril in dosage forms was accurate within the acceptance level of 95% to 100%.
(Table 8).

Table 8: Assay results of valsartan and sacubitril in formulations [Azmarda-50]

Drug Name	**Quantity Label Claim(mg)**	***Quantity Found ± SD**	**% Assay ± SD**	***%RSD**
Valsartan	26	25.97 ± 0.08	99.88 ± 0.04	0.030
Sacubitril	24	24.29 ± 0.11	101.21±0.07	0.027

*Average of six determinations

APPLICATIONS

This method can be used for the quality control of valsartan and sacubitril in combined dosage forms within a short analysis time.

CONCLUSIONS

The proposed method describes the development of a selective, accurate and sensitive RP-HPLC method for the simultaneous estimation of valsartan and sacubitril in pure and marketed formulations with good resolution.The validation results demonstrated that the proposed RP-HPLC procedure is suitable for the intended purpose and can be employed easily used for the routine quality control of valsartan and sacubitril in combined dosage forms within a short analysis time.

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