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Reverse Phase Stability Indicating HPLC Method for Determination of Sacubitril and Valsartan in the Presence of its Stress Degradation Products

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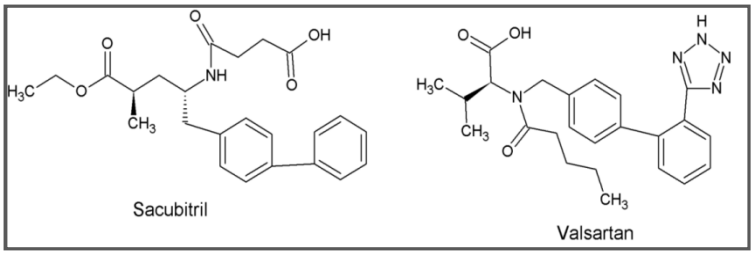
ABSTRACT

A rapid, sensitive reversed phase stability indicating high performance liquid chromatographic method was developed and validated for the simultaneous assay of sacubitril and valsartan in bulk and tablet dosage forms. The method was developed using the Sunsil C18 analytical column using isocratic elution with mobile phase consisting of 0.1M dipotassium hydrogen phosphate and methanol in the ratio of 65:35 (v v^{-1}) at a flow rate of 1.0 mL min^{-1}. Sacubitril and valsartan were monitored at 254 nm. These were subjected to various stress conditions of acid, base, oxidative, thermal and photolytic degradations. The method is efficient for the estimation of sacubitril and valsartan in the presence of stress degradation products. The method performance was validated according to the ICH guidelines for selectivity, specificity, limit of detection, limit of quantification, linearity, accuracy, precision and robustness. The results of validation parameters are found to be within the recommended limits. Therefore, the method is appropriate for stability study and quantification of sacubitril and valsartan in tablet samples.

Graphical Abstract



Keywords: Sacubitril, Valsartan, Heart Failure, Stability Indicating, Assay.

INTRODUCTION

Valsartan, chemically known as (*S*)-3-methyl-2-(*N*-{[2'-(2*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl] methyl} pentanamido) butanoic acid, is an orally active nonpeptide, effective and specific competitive

antagonist of the angiotensin-II AT1receptor belonging to the class of compounds known as biphenyltetrazoles and derivatives [1]. Valsartan is used in treating high blood pressure, heart failure, decreasing the risk of death after a heart attack in patients with left ventricular dysfunction [2]. Valsartan promotes vasodilation and decreases the effects of aldosterone by blocking the angiotensin II binding to AT1 receptors [3, 4].

Sacubitril, chemically described as 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid, is a prodrug and inhibits the enzyme, neprilysin [5]. Sacubitrilat, active metabolite of sacubitril, inhibits neprilysin which cleave natriuretic peptides like atrial natriuretic peptide, brain natriuretic peptide and c-type natriuretic peptide. Also, neprilysin degrade vasodilating peptides and vasoconstrictors like angiotensin I and II. Therefore inhibition of neprilysin results in decreased breakdown and increased concentration of natriuretic peptides and increased levels of angiotensin I and II.

The combination of sacubitril with valsartanis approved by FDA in 2015 to treat heart failure patients with decreased ejection fraction [6, 7]. The chemical structure of valsartan and sacubitril are shown in figure 1.

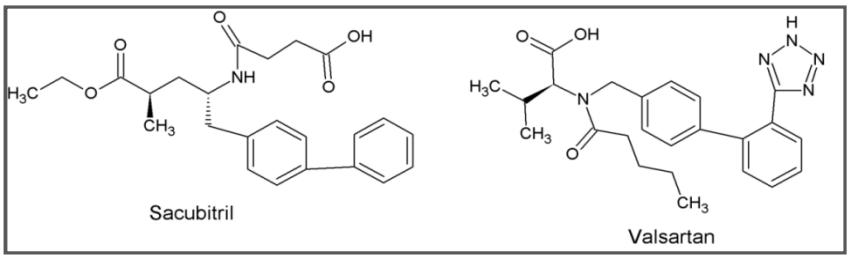


Figure 1. Chemical structure of studied drugs.

There have been few reports for the combined determination of sacubitril and valsartan in pharmaceutical formulations and rat plasma including UV spectrophotometry [8, 9], spectrofluorimetry [10], high performance liquid chromatography [11-17], ultra performance liquid chromatography [18] and liquid chromatography with tandem mass spectrometry [19]. Though the UV Spectrophotometric methods [8, 9] are simple, they lack selectivity as they involve measurements in UV region where most of the excipients show absorption. The spectrofluorimetry [10], ultra performance liquid chromatography [18] and liquid chromatography with tandem mass spectrometry [19] are sensitive enough but they require costly and sophisticated equipment. Furthermore, the liquid chromatography with tandem mass spectrometry [19] is not applied to tablet dosage form. Although some high performance liquid chromatography methods were reported for the same purpose, they suffer from disadvantages like narrow range of linearity [11, 14], less precise [11-15, 17], less accurate [11-13, 16], use of triple solvent system as mobile phase [12, 13, 15, 16], less sensitive [12, 15-17], more runtime [13-15, 17]. Some of the HPLC methods are not fully validated [11, 14, 15, 17]. Few HPLC methods are not stability indicating methods [11-13]. Though the remaining HPLC methods are stability indicating, peak purity details are not reported [14, 16, 17].

The present report describes a rapid, sensitive and selective stability indicating HPLC method for the simultaneous quantification of sacubitril and valsartan. The method was validated according to the International Conference on Harmonization guideline [20] and applied for the analysis of sacubitril and valsartan in tablets.

MATERIALS AND METHODS

Materials: Reference standards of sacubitril and valsartan reference were obtained as gift samples from Lara drugs pvt Ltd., Hyderabad. Methanol was HPLC grade and obtained from Merck India

Ltd., Mumbai. Analytical regent grade hydrogen peroxide, hydrochloric acid, sodium hydroxide,dipotassium hydrogen phosphate and orthophoshoric acid were from Sd. Fine Chemicals Ltd., Mumbai, India. Entresto tablets (each containing 97 mg sacubitril and 103 mg valsartan) were purchased from Novartis Pharmaceuticals, India. Milli-Q water (Millipore, USA) was used throughout the analysis.

Mobile phase: Mobile phase was prepared by mixing 0.1 M K_2HPO_4 and methanol (65:35, *v/v*). 0.1M dipotassium hydrogen phosphate buffer was prepared by dissolving 17.45 g of dipotassium hydrogen phosphate in 300 mL of double distilled water in a 1000 mL volumetric flask and made up to the volume with the same water. The pH of mobile phase was adjusted to 3.5 with orthophosphoric acid. Prior to use, mobile phase was filtered using 0.45 µm pore size membrane filter and degassed by sonication for 15 min.

Instrumentation: The HPLC system was a Waters 2695 alliance with binary HPLC pump, degasser, autosampler, and Waters 2998 photodiode array detector. Waters Empower 2 software was used for analysis. Sunsil C18 (250 × 4.6 mm; 5 µm particle size) analytical column was used for separation and analysis. Electronic balance (ELB 300) for weighing the materials and Digisun pH meter for pH measurements was used.

Chromatographic conditions: Column temperature was set at 25°C. The mobile phase flow rate was 1 mL min^{-1} and injection volume of 10 µL. The studied drugs were detected and quantified using photodiode array detector set at 254 nm. The run time was 6 min and the peak areas of studied drugs were used to quantify them.

Standard solutions: Stock solutionof sacubitril and valsartanat 970 µg mL^{-1} and 1030 µg mL^{-1}, respectively was prepared in 100 mL of mobile phase using reference standards of sacubitril (97 mg) and valsartan (107 mg). Further dilutions to obtain working standard solutions were made in mobile phase. Working standard solutions for calibration curve were prepared by serial dilution containing 48.5, 72.5, 97.0, 121.25, 145.5 µg mL^{-1} sacubitril and 51.5, 77.25, 103.0, 128.75, 154.50 µg mL^{-1} valsartan. Working standard solutions for validation parameters (system suitability, selectivity, precision, accuracy and robustness) were prepared at a concentration of 97.0 µg mL^{-1} and 103.0 µg $mL^{-1}$of sacubitril and valsartan, respectively.

General assay procedure: Working standard solutions containing 48.5, 72.5, 97.0, 121.25, 145.5 µg mL^{-1} of sacubitril and 51.5, 77.25, 103.0, 128.75, 154.50 µgmL^{-1} of valsartan were prepared using mobile phase. An aliquot of 10 µL of each working standard solution was injected into the chromatographic system and processed according to the previously described chromatographic conditions. From the peak area response and concentration of studied drugs, calibration curve was constructed. The calibration data was treated using least-squares regression analysis.

Tablet sample solution: Stock solution of tablet sample at 970 µg mL^{-1} and 1030 µg mL^{-1} concentration of sacubitril and valsartan respectively were prepared in mobile phase. For this purpose, 10 Entresto tablets (each containing 97 mg sacubitril and 103 mg valsartan) were weighted and finely powdered with mortar. Appropriate amount powder equivalent to concentration of sacubitril and valsartan in one tablet was dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. This mixture was sonicated for 30 min and filtered using 0.45 µm pore size membrane filter. The resulting solution was made up to 100 mL with mobile phase. This tablet sample stock solution was aptly diluted to a concentration of 97.0 µg mL^{-1} and 103.0 µg mL^{-1} of sacubitril and valsartan respectively for the analysis.

Assay of valsartan and sacubitril in tablets: An aliquot of 10 µL of tablet sample solution (97.0 µg mL^{-1} - sacubitril and 103.0 µg mL^{-1} - valsartan) was injected into the chromatographic system and processed according to the previously described chromatographic conditions. The peak area response

of sacubitril and valsartan was then used to calculate the content of the studied drugs in tablets using corresponding calibration curve or regression equation.

Stress degradation study: The tablet sample was subjected to stress degradation under different stress conditions like acidic, basic, oxidative, thermal and photo stress conditions following International Conference on Harmonization guideline [21]. The stock solution of stress degradation sample was prepared as follows:

Acid stress degradation: An accurately weighed amount of tablet powder (97 mg sacubitril and 103 mg valsartan) was dissolved in 10 mL of 0.1N HCl in 100 mL volumetric flask and sonicated for 30 min at room temperature. The solution was then neutralized with 0.1N NaOH and volume was then completed using mobile phase.

Base stress degradation: An accurately weighed amount of pure (97 mg sacubitril and 103 mg valsartan) was transferred to a 100 mL volumetric flask, 10 mL of 0.1N NaOH was added and sonicated at room temperature for 30 min. The solution was neutralized with 0.1N HCl and completed to volume using mobile phase.

Oxidative stress degradation: Tablet powder equivalent to 97 mg of sacubitril and 103 mg of valsartan was transferred to a 100 mL volumetric flask followed by addition of 10 mL of 3% H_2O_2 and sonicated at room temperature for 30 min. The solution was then made upto volume with mobile phase.

Thermal stress degradation: Tablet powder equivalent to 97 mg of sacubitril and 103 mg of valsartan was kept in oven at 105°C for 30 min. The powder was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. This mixture was sonicated for 30 min and made upto the mark with mobile phase.

Photo stress degradation: Tablet powder equivalent to 97 mg of sacubitril and 103 mg of valsartan was kept in direct sunlight for 24 hr. The powder was cooled and dissolved in 30 mL of mobile phase in a 100 mL volumetric flask. This mixture was sonicated for 30 min and made upto the mark with mobile phase.

The stock solution of stress degradation samples were diluted aptly with mobile phase to produce a solution with concentration of 97.0 µg mL^{-1} and 103.0 µg mL^{-1} sacubitril and valsartan, respectively. The diluted solutions were filtered using 0.45 µm pore size membrane filter and injected (10 µL) into the HPLC system. The percentage degradation of valsartan and sacubitril was calculated in all the applied stress conditions. Peak purity of valsartan and sacubitril peaks was verified by using the photodiode array detector in the stress samples.

RESULTS AND DISCUSSION

Method optimization: The main goal of the present method is to achieve the good separation and analysis of sacubitril and valsartan in the presence of their stress degradation products. The optimization of chromatographic conditions was done using two different analytical columns, mobile phase compositions, mobile phase flow rate, mobile phase pH and column temperatures. Zorbax XDB C18 column (250 × 4.6 mm; 5 µm particle size) and Sunsil C18 (250 × 4.6 mm; 5 µm particle size) column with different temperatures (25°C, 30°C, 35°C) were tried as stationary phase. Good resolution and better peaks were obtained with Sunsil C18 (250 × 4.6 mm; 5 µm particle size) and temperature of 25°C. Hence the same column and temperature was used. 0.1M dipotassium hydrogen phosphate and methanol in different ratios, pHs (3, 3.5, 4, 4.5,) and flow rates (0.8, 1.0, 1.2) were tested. Shorter retention times with appropriate peak shapes and peak area response were observed with mobile phase consisting of 0.1M dipotassium hydrogen phosphate and methanol in the ration of 65:35 ($v\ v^{-1}$) with pH 3.7 and at a flow rate of 1.0 mL min^{-1}. therefore the same conditions were

selected for the analysis. 254 nm, the wavelength where maximum peak area response of both the drugs is observed, is chosen as analytical wavelength. A chromatogram of sacubitril and valsartan obtained at optimum chromatographic conditions is shown in figure 2.

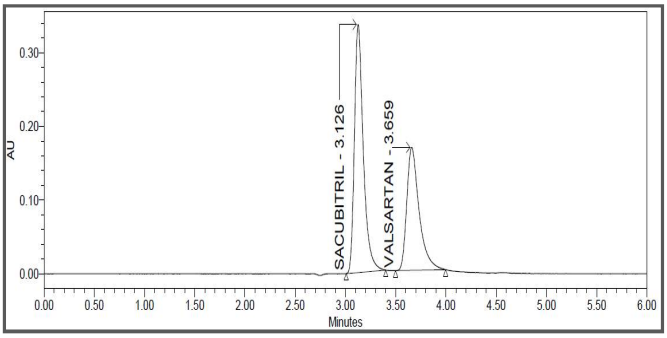


Figure 2. Chromatogram of sacubitril and valsartan obtained at optimum chromatographic conditions.

Method validation: Validation of the developed method was done according to the International Conference on Harmonization guideline [**20**]. The method was validated for system suitability, selectivity, linear range, accuracy, precision, detection and quantification limits, specificity and robustness.

System suitability was checked by relative standard deviation of peak area and retention time, theoretical plates, resolution and tailing factor for the peaks of sacubitril and valsartan. As shown in table 1, the system was found to be suitable relative to the recommended limits.

Table 1. System suitability results of the proposed method

Parameters	Sacubitril	Valsartan	Recommended limits
Retention time	3.122 (%RSD – 0.100)	3.650 (%RSD – 0.123)	RSD ≤2
Peak area	2076342 (%RSD – 0.521)	1430971.400 (%RSD – 0.532)	RSD ≤2
USP resolution	-	2.686	> 1.5
USP plate count	6206	4431	> 2000
USP tailing factor	1.396	1.534	≤ 2

All the values given the table are average of five determinations

The chromatograms of mobile phase blank (Fig. 3A), placebo blank (Fig. 3B), working standard solution (Fig. 3C) and tablet sample solution (Fig. 3D) were compared to assess the selectivity of the method. From Fig.3C and 3D, the retention times of sacubitril and valsartan in working standard solution and tablet sample solution were similar. No peaks at the retention time of sacubitril and valsartan were observed in the mobile phase blank and placebo blank chromatograms. The results indicated the selectivity of the method.

The linearity range for sacubitril and valsartan was found to be in the concentration range of 48.5-145.5 µg mL^{-1} and 51.5-154.50 µg mL^{-1}, respectively. Linearity was estimated by linear regression analysis using the least squares regression method. The regression equation obtained was:

For sacubitril: $y = 21421\ x - 3083$ ($R^2 = 0.9997$)
For valsartan: $y = 13903\ x - 459.2$ ($R^2 = 0.9998$)

Where y is peak area response of drugs, x is concentration of drugs in µg mL^{-1} and R^2 is regression coefficient. The results confirmed the good linear relationship between peak area responses and concentrations of drugs.

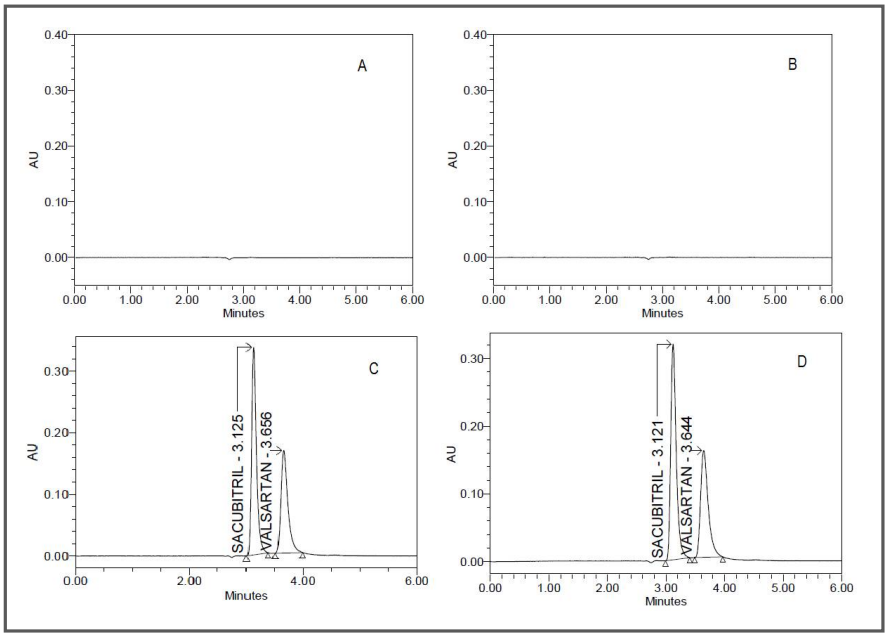


Figure 3. Chromatogram of [A] Mobile phase blank [B] Placebo blank [C] Standard solution [D] Tablet sample solution.

The limits of detection (LOD) and quantification (LOQ) for sacubitril and valsartan were assessed at a signal tonoise ratio of 3:1 and 10:1, respectively. The LOD for sacubitril and valsartan was 0.604 µg mL^{-1} and 1.054 µg mL^{-1} respectively. The LOQ of sacubitril and valsartan was found to be 2.012 µg mL^{-1} and 3.515 µg mL^{-1} respectively.

The precision of the method was verified by repeatability. Six replicate working standard solutions were prepared analyzed by the proposed method. The average peak area response and % RSD of the peak area response for sacubitril and valsartan was calculated for precision. The results (%RSD <2%) confirmed the precision of the method (Table 2).

Table 2. Precision and accuracy results of the proposed method

Sample No.	Sacubitril		Valsartan	
	Peak area response	Assay (%)	Peak area response	Assay (%)
1	2076266	99.4	1437605	100.06
2	2071799	99.18	1432515	99.71
3	2076539	99.41	1439927	100.22
4	2070360	99.11	1431450	99.63
5	2071760	99.18	1434955	99.88
6	2071028	99.15	1438419	100.12
Average	2072959	99.24	1435812	99.94
RSD	0.131	0.133	0.236	0.236

For accuracy determination, six replicate working standard solutions were prepared analyzed by the proposed method. The % assay of sacubitril and valsartan was calculated. The results (~100%) confirmed the accuracy of the method (Table 2).

The method accuracy was also evaluated by analyzing the placebo, in triplicate, spiked with reference standards of sacubitril and valsartan at three different concentration levels (50%, 100% and 150%). The percent recovery at each concentration level was determined. As seen in table 3, good recovery values (~100%) were obtained. No interference caused by common excipients was observed, indicating the accuracy and selectivity of the method.

Table 3. Recovery results of the proposed method

Spiked Level (%)	Concentration of drug (µg mL^{-1})		Recovery (%)	Mean (%)
	Spiked	Found		
	Sacubitril			
50	48.015	47.91	99.79	
	48.015	47.97	99.91	100.02
	48.015	48.19	100.36	
100	96.030	96.40	100.38	
	96.030	96.14	100.12	100.25
	96.030	96.27	100.25	
150	144.045	144.49	100.31	
	144.045	144.57	100.37	100.40
	144.045	144.81	100.53	
	Valsartan			
50	51.50	51.31	99.64	
	51.50	51.31	99.64	99.64
	51.50	51.32	99.65	
100	103.00	103.01	100.01	
	103.00	103.17	100.16	100.12
	103.00	103.20	100.20	
150	154.50	153.75	99.51	
	154.50	153.60	99.42	99.46
	154.50	153.67	99.46	

To prove the specificity and stability indicating nature of the developed method, tablet sample was exposed to acid, base, peroxide, thermal and photolytic stress conditions. The results of stress degradation are reported in table 4. Both the drugs showed degradation in all the applied conditions. More percentage of sacubitril and valsartan degradation was observed in photolytic condition. Sacubitril and valsartan showed less degradation in alkaline and acid conditions, respectively. Overall valsartan was more stable to the applied degradation conditions in comparison to sacubitril. The chromatograms of all the degraded samples are shown in fig. 4-8. In the entire degradation conditions only one degradant peak was observed.

Peak purity of valsartan and sacubitril peaks was verified in the stress samples. Peak is pure only when purity threshold is greater than purity angle. From the data given in table 4, it was observed that the peaks of valsartan and sacubitril are pure in all the stress samples. The purity and assay of valsartan and sacubitril was unaffected by the presence of their degradation products. This confirms the specificity and stability indicating power of the method.

Table 4. Specificity and stability data of the proposed method

Stress condition	Drug	Peak area	Assay (%)	Degra-dation (%)	Purity angle	Purity threshold	Retention time of degradants
Undegraded	SAC	3780471	100	-	-	-	-
	VAL	6528785	100	-	-	-	
Acid	SAC	3243592	85.28	14.72	0.292	0.765	2.735
	VAL	5936997	90.57	9.43	0.204	0.468	
Alkaline	SAC	3333568	87.65	12.35	0.421	0.860	2.741
	VAL	5866458	89.5	10.50	0.195	0.336	
Oxidative	SAC	3193289	83.96	16.04	0.312	0.931	2.741
	VAL	5789812	88.33	11.67	0.116	0.285	
Thermal	SAC	3263658	85.81	14.19	0.526	1.065	2.739
	VAL	5696341	86.9	13.10	0.196	0.365	
Photo	SAC	3176895	83.53	16.47	0.29	0.727	2.737
	VAL	5626972	85.84	14.16	0.186	0.385	

SAC - Sacubitril; VAL- Valsartan

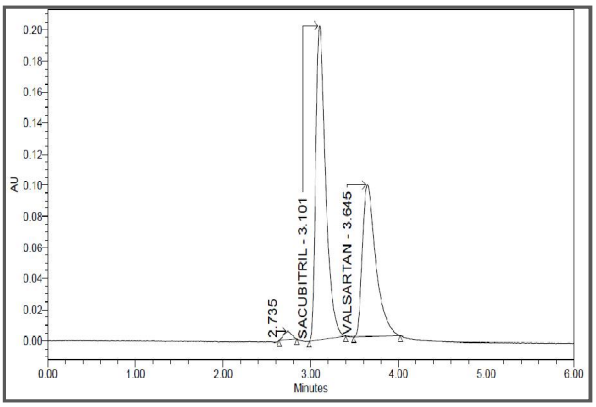


Figure 4. Typical chromatogram of sacubitril and valsartan forced degradation study–Acid degradation.

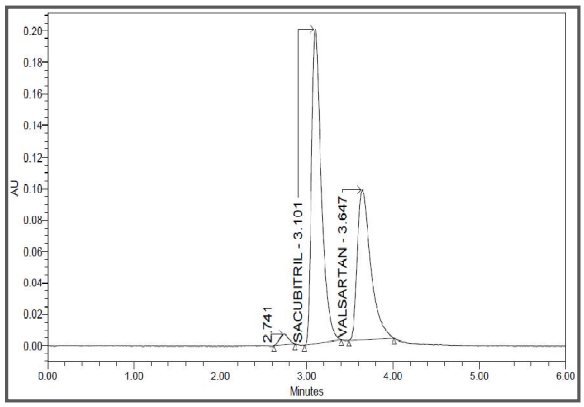


Figure 5. Typical chromatogram of sacubitril and valsartan forced degradation study–Base degradation.

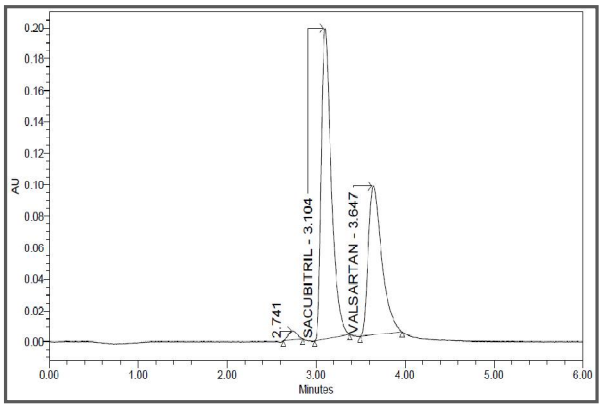


Figure 6. Typical chromatogram of sacubitril and valsartan forced degradation study–Oxidative degradation.

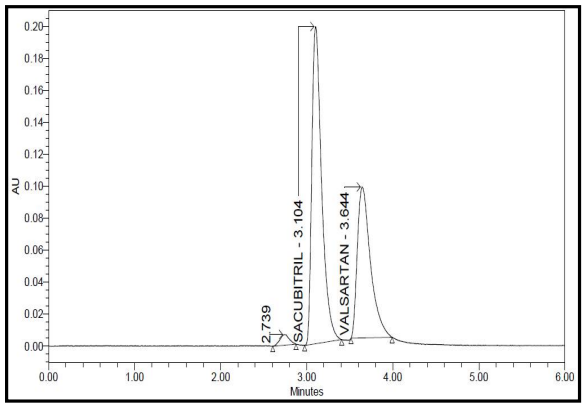


Figure 7. Typical chromatogram of sacubitril and valsartan forced degradation study – Thermal degradation.

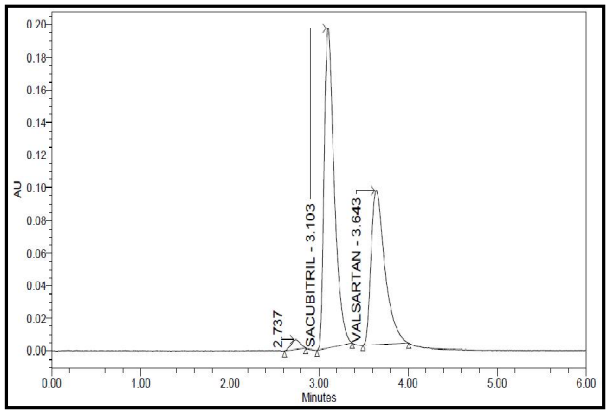


Figure 8. Typical chromatogram of sacubitril and valsartan forced degradation study–Photo degradation.

To find out the robustness of the method, chromatographic conditions were deliberately changed and the retention time, peak area response, plate count, tailing factor and resolution for sacubitril and valsartan. The flow rate of the mobile phase was changed by varying 0.1 unit and verified at 0.9 and 1.1 mL min^{-1}. The effect of the column temperature was studied at 23°C and 27°C instead of at 25°C. In all the studied chromatographic conditions, no significant changes were observed in the studied parameters (Table 5). The results indicated the robustness of the method.

Table 5. Robustness data of the proposed method

Parameter varied	Retention time	Peak area	Plate count	Tailing factor	Resolution
		Sacubitril			
Flow rate – 0.9 mL min^{-1}	2.585	1706394	4447	1.37	-
Flow rate – 1.1 mL min^{-1}	3.892	2593791	6230	1.42	-
Column temperature-23°C	2.585	1691461	4319	1.37	-
Column temperature-27°C	3.888	2591384	6128	1.40	-
		Valsartan			
Flow rate – 0.9 mL min^{-1}	3.015	1166535	3148	1.51	2.29
Flow rate – 1.1 mL min^{-1}	4.494	1758852	4216	1.53	2.44
Column temperature-23°C	3.023	1160898	3106	1.54	2.29
Column temperature-27°C	4.488	1770765	4117	1.54	2.42

APPLICATION

The present method is stable and can be used for the routine analysis of tablet samples of sacubitril and valsartan.

CONCLUSION

The rapid stability indicating RP-HPLC method was developed and validated for the determination of sacubitril and valsartan in bulk and tablet dosage forms. The developed method was found to be selective, precise, accurate, linear, robust and specific. The method can separate sacubitril and valsartan from their degradation products. The method is stability indicating and can be used for the routine analysis of tablet samples and to check the stability of sacubitril and valsartan samples.

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