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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and reproducible Reverse Phase High Performance Liquid Chromatography method was developed and validated for simultaneous estimation of Sacubitril and Valsartan in tablet dosage form. Chromatographic separation was achieved by Grace C_{18} (250 mm x 4.6 ID, Particle size- 5 micron) column and methanol: water (90:10v/v) as mobile phase, at a flow rate of 1 ml/min (millilitre per minute) using UV detection at 244nm. Forced degradation experiments were carried out by exposing Sacubitril and Valsartan standard and sample for thermal, photolytic, oxidative and acid-base hydrolytic stress conditions. The retention time for Sacubitril and Valsartan were obtained as 6.984min and 5.311 min. respectively. The method has been validated for linearity, accuracy, precision, LOD, and LOQ. Linearity of Sacubitril and Valsartan were found to be 12-60µg/ml.(R^2=0.9987) and 13-65µg/ml.(R^2=0.9979) respectively. The accuracy of present method was evaluated at 50%, 100%, 150%. Recovery was found to be in a range from 99.13%-101.25% for sacubitril and 98.92%-101.80% for valsartan. Intermediate precision studies were carried out and the RSD values were less than 2%. Lower values of LOD (0.096µg/ml) and LOQ (0.293µg/ml) for sacubitril and LOD (0.280µg/ml) and LOQ (0.849µg/ml) for valsartan indicated good sensitivity of the method. In this study, the optimization of mobile phase, flow rate, injection volume and wavelength were achieved. This demonstrate that the developed method is simple, precise, accurate and robust for simultaneous estimation of Sacubitril and Valsartan in tablet dosage form. The

method was acceptable for degradation studies of heat, sunlight, acid, base, peroxide which meet the acceptance criteria for forced degradation studies.

KEYWORDS: Sacubitril, Valsartan, RP-HPLC, Validation.

INTRODUCTION

Sacubitril is a prodrug that is activated to sacubitrilat by de-ethylation via esterases. Sacubitrilat inhibits the enzyme neprilysin, which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume.[1-3]

Valsartan is an angiotensin II receptor antagonist (commonly called an ARB, or angiotensin receptor blocker), blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure. The drug binds to angiotensin type I receptors (AT1), working as an antagonist. This mechanism of action is different than the ACE inhibitor drugs, which block the conversion of angiotensin I to angiotensin II.[4-6]

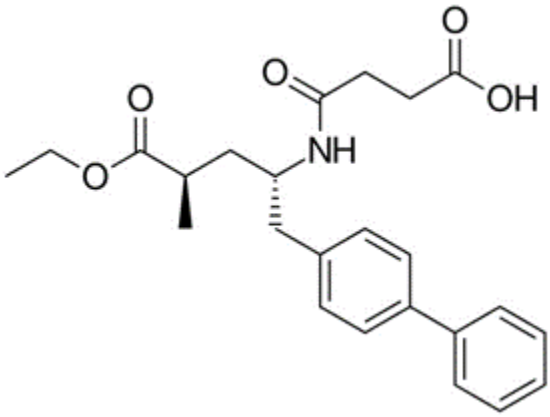


Fig. 1: Chemical structure of Sacubitril.

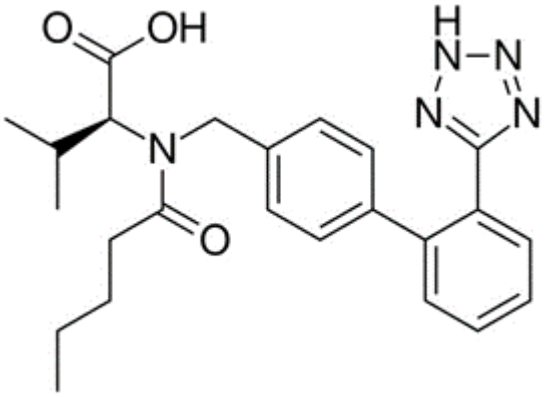


Fig. 2: Chemical structure of Valsartan.

On literature survey, it was found that only few methods was found for the simultaneous estimation of Sacubitril and Valsartan in combined dosage forms and no method is available in the pharmacopoeias. Few analytical methods like UV, RP-UPLC have been developed for the determination of Sacubitril and Valsartan individually and in combination with other drugs.[7-14] So we have developed a novel, simple and highly sensitive stability indicating RP-HPLC methods for Sacubitril and valsartan in bulk and pharmaceutical formulations and validated according to ICH guidelines.

MATERIALS AND METHOD

List of Instruments

Table 1: List of apparatus/ instruments used.

Sr. no.	Name	Model	Manufacturer/Supplier
1.	Weighing balance	PGB 100 Max : 100gm Min : 0.001gm	Wenser High Precision Balance
2.	Digital PH Meter	PICO+	Lab India pvt ltd.
4.	Sonicator	WUC-4L Capacity -4 liter	Wenser Ultra Sonicator
5.	Magnetic stirrer		Remi Equipment
6.	HPLC	HPLC 3000 Series	Analytical Technologies Ltd.

Table 2: HPLC Instrument Information.

Parts of Instruments	Information
System	HPLC Binary Gradient System
Model no.	HPLC 3000 Series
Company	Analytical Technologies Ltd.
Pump	P-3000-M Reciprocating (40 MPa)
Column	Grace C18 (250mm×4.6ID, Partical size- 5 micron)
Detector	UV-3000-M
Software	HPLC Workstation

List of Chemicals

Table 3: List of chemical used.

Sr.No.	Name	Specification	Manufacturer/Supplier
1.	Methanol	HPLC grade	Merck
2.	Purified water	HPLC grade	In house production
3.	0.1N HCL	HPLC grade	Merck
4.	0.1N NaOH	HPLC grade	Merck
5.	3% H_2O_2	HPLC grade	Merck

Table 4: List of API used.

Sr. No.	Name	Specification	Manufacturer/Supplier
1.	Sacubitril	Working standard	Lupin ltd. India.
2.	Valsartan	Working standard	Lupin ltd. India.

Preparation of mobile phase

Mixed a HPLC grade Methanol and Purified water (90:10) in volumetric flask. Filter through 0.45µ filter under vaccum filtration.

Diluent preparation: Use mobile phase as Diluent.

Preparation of standard solutions

Accurately weigh and transfer 0.01gm (10mg) of pure sacubitril and valsartan working standard separately into 10ml clean and dry volumetric flask. Add diluent and sonicated to dissolve it completely and made volume upto the mark with same solvent (mobile phase). From this, five working standard solution of concentration covering the range 12-60 ppm and 13-65 ppm for sacubitril and valsartan respectively, were prepared by transferring and diluting different aliquots into series of 10ml volumetric flask with same diluent.

Preparation of sample solutions

Weighed and transfer 20 tablets of Vymada 50mg [containingsacubitril 24mg and valsartan 26mg lable claim] into mortar and pestle. Crush the above tablets into fine powder. Weigh and transfer sample powder quantity equivalent to 10 mg of sacubitril and valsartan in 10 ml volumetric flask containing mobile phase and shaken vigorously, sonicated for 15 min and made up volume up to the mark with mobile phase. Aliquots of the above solution was pipetted and transferred into a series of clean and dry 10 ml volumetric flask and diluent was added uo to the mark to get final concentration of sacubitril and valsartan. 20µL volume each of these standard and sample solution were injected five times and the peak areas were recorded.

Selection of wavelength: UV spectrum of 10µg/ml sacubitril and valsartan diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the spectrum wavelength selected as 244nm. At this wavelength, both drugs show good absorbance.

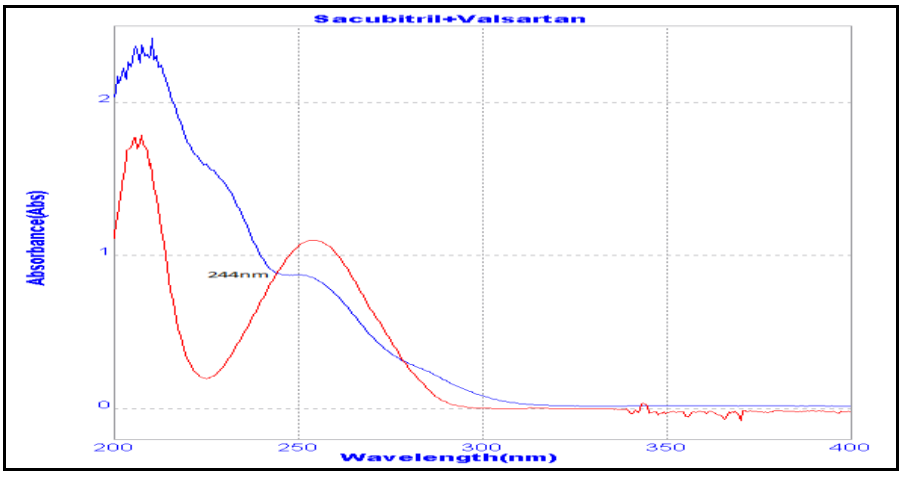


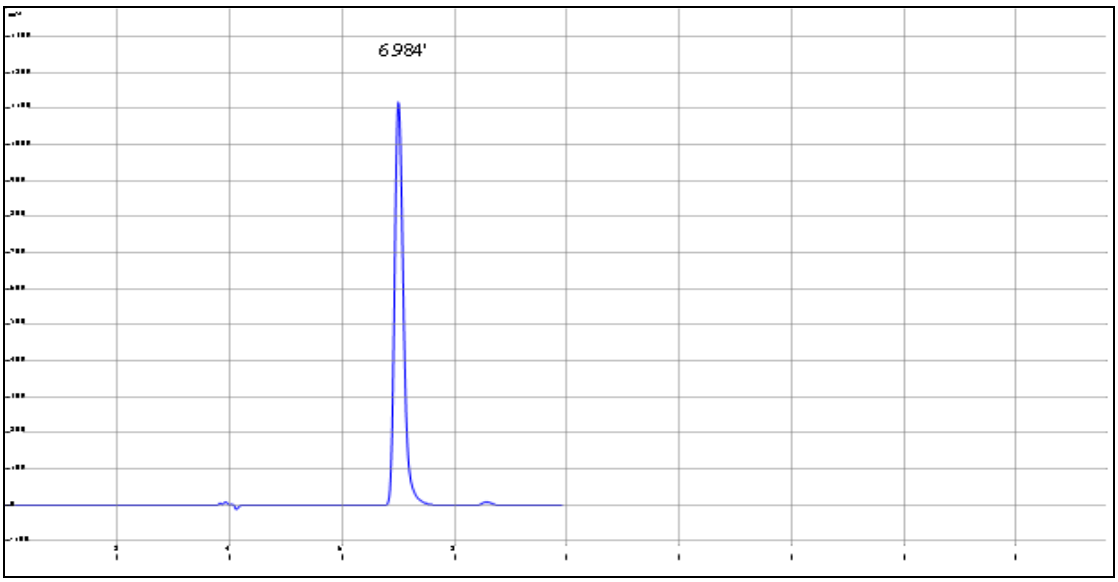
Fig. 3: Wavelength of Sacubitril and Valsartan.

HPLC instrumentation and chromatographic conditions

The HPLC system was Binary Gradient system consisting of pump P-3000-M Reciprocating (40MPa), detector UV-3000-M, column Grace C18 (250mm×4.6ID, Particle size- 5micron), thermo scientific injector rheodyne injector (20µl capacity) and syringe Hamilton (25µl). Data were processed using HPLC workstation software. A freshly prepared mixture of methanol and purified water (90:10 v/v) (PH 3.0) used as the mobile phase. Mobile phase was filtered through 0.45µm membrane filter and sonicated before used. The flow rate of mobile phase was maintained at 1.0 ml/min. The eluents were monitored at 244nm. The injection volume of sample and standard were 20µl. Total run time is 10 min.

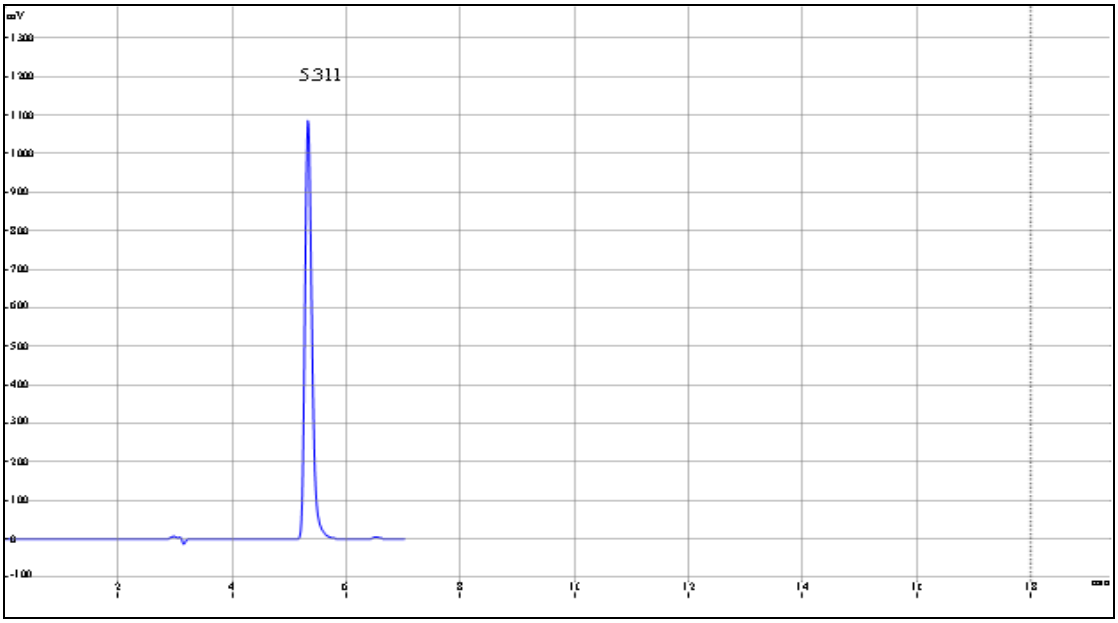
Optimized chromatographic condition

In the present study the separation of sacubitril and valsartan was achieved by using C18 column Grace C18 (250mm×4.6ID, Particle size- 5micron) with mobile phase consisting of mixture of methanol and water in the ratio of 90:10 at a flow rate 1.0 ml/min with uv detection wavelength of 244nm at ambient temperature. The retention time for sacubitril and valsartan were found to be 6.984 and 5.311min respectively (fig no 4 and 5).



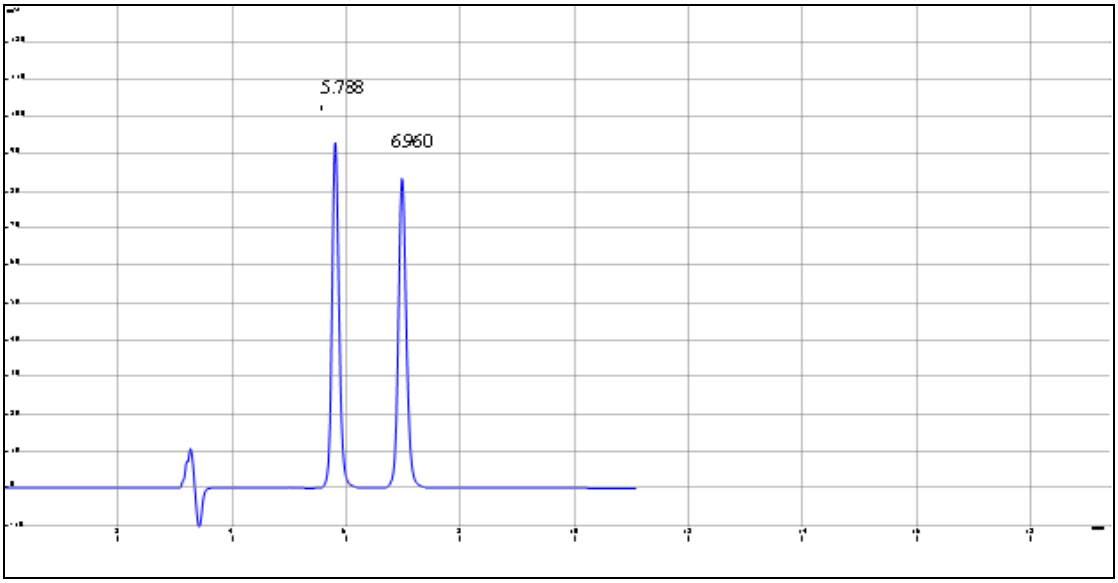
Time	Conc	Area	Resolution	T.Platenum	Asymmetry
6.984	24ppm	13097587	0.00	8456	1.32

Fig. 4: Retention time of SAC.



Time	Conc	Area	Resolution	T.Platenum	Asymmetry
5.311	26ppm	9773330	0.00	8326	1.31

Fig. 5: Retention time of VAL.



Time	Conc	Area	Resolution	T.Platenum	Asymmetry
5.788	26ppm	928676	3.56	6162	1.14
6.960	24ppm	987012	0.00	7337	1.13

Fig. 6: Chromatogram of standard solution for SAC and VAL.

RESULT AND DISCUSSION

Method Validation

The developed method was validated as per ICH guidelines for its specificity, system suitability, linearity, accuracy, precision, robustness, limit of detection, limit of quantification.

System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and the tailing factor was calculated. The results are given in table 5.

Table 5: System suitability parameters for sacubitril and valsartan.

System suitability parameters	Sacubitril	Valsartan
Retention time	6.960	5.788
Theoretical plate no.	987012	928676
Tailing factor	1.13	1.14
Resolution		3.56

Linearity

The linearity of this method was evaluated by linear regression analysis and calculated by the least square method and studied by preparing standard solutions of sacubitril and valsartan at different concentration levels. The calibration curve showed (Fig. 7 and 8) good linearity in the range of 12.25-36.75 µg/ml, for sacubitril with a correlation coefficient (r2) of 0.999 and 12.75-38.25 µg/ml for valsartan with a correlation coefficient (r2) of 0.999. Results are given in table 6.

Table 6: Linearity data for sacubitril and valsartan.

Drug	Concentration (ppm)	Area
Sacubitril	12	343325
	24	987012
	36	1633733
	48	2337416
	60	3095238
Valsartan	13	476503
	26	928676
	39	1567013
	52	2064676
	65	2648219

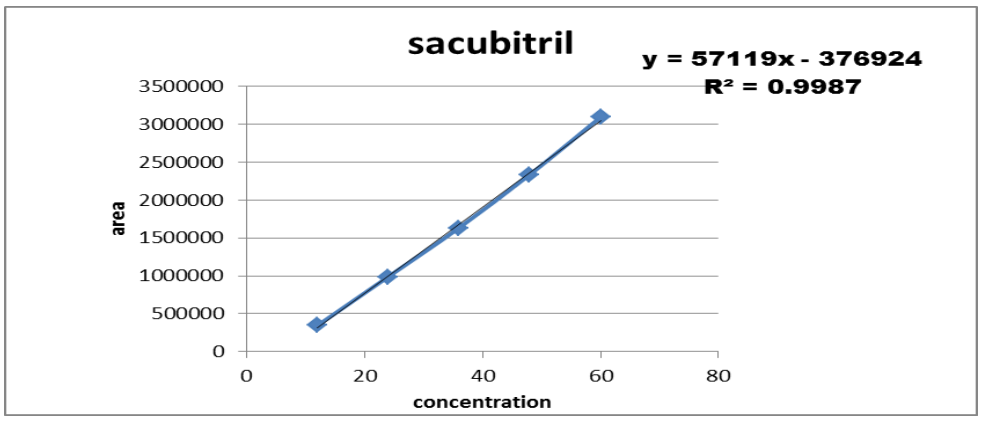


Fig. 7: Linearity graph of Sacubitril.

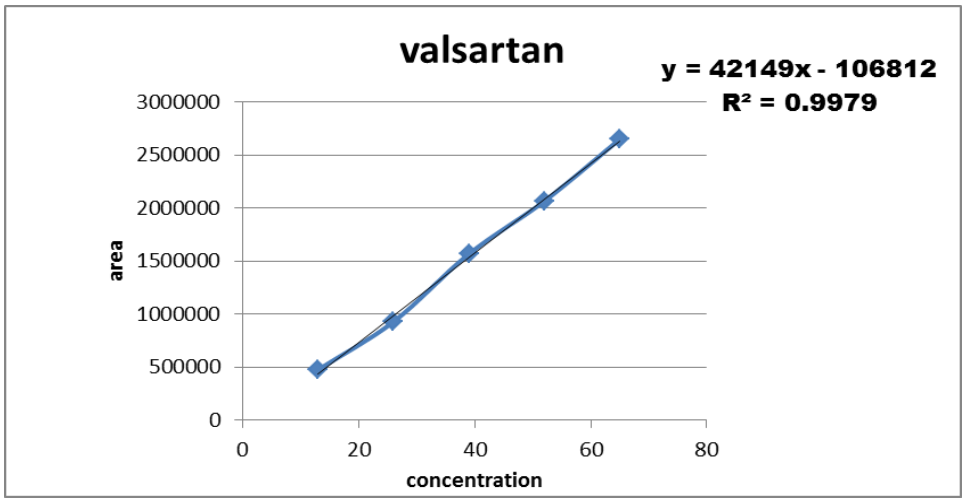


Fig. 8: Linearity graph of Valsartan.

Accuracy

Recovery studies were carried out by addition of the standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. At each concentration, the sample was injected thrice to check repeatability and from the % RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.13-101.25% for the Sacubitril and 98.92-101.80% for valsartan at three different concentrations 80%, 100%, 120%. The results are given in table 7 and 8.

Table 7: Accuracy data for sacubitril.

Conc (%)	Sampleamount (ppm)	Amount added (ppm)	Amount recovered(ppm)	% recovery	%mean recovery
50%	24	12	36.18	100.52	100.64
	24	12	36.17	100.47	
	24	12	36.33	100.93	
100%	24	24	47.50	98.50	99.13
	24	24	47.47	99.89	
	24	24	47.53	99.02	
150%	24	36	60.88	101.36	101.25
	24	36	60.76	101.27	
	24	36	60.68	101.14	

Table 8: Accuracy data for valsartan.

Conc (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered(ppm)	% recovery	%mean recovery
50%	26	13	39.77	101.99	101.8
	26	13	39.66	101.71	
	26	13	39.66	101.70	
100%	26	26	51.41	98.86	98.92
	26	26	51.48	99.00	
	26	26	51.43	98.90	
150%	26	39	65.33	100.51	100.50
	26	39	65.31	100.48	
	26	39	65.34	100.53	

Precision

Repeatability

A standard solution containing sacubitril (12 µg/ml) and valsartan (13 µg/ml) was injected three times and areas of peaks were measured and % RSD was calculated. The results are given in table 9.

Table 9: Repeatability data for sacubitril and valsartan.

Drug	Conc (ppm)	Area	Mean ±SD (n=3)	% RSD
Sacubitril	12	343325	343540.66	0.329
		342555		
		344742		
Valsartan	13	476503	477041.66	0.314
		475883		
		478739		

Intraday precision

A standard solution containing (12,24,36 µg/ml) of sacubitril and (13, 26,39 µg/ml) of valsartan were analyzed three times on the same day and % R. SD was calculated. The results are given in table 10.

Table 10: Intraday data for sacubitril and valsartan.

Sacubitril		Valsartan	
	Area		**Area**
Morning	1633733	**Morning**	1567013
	1633499		1565224
	1632141		1565091
Evening	1635909	**Evening**	1565479
	1631995		1566367
	1635125		1565501
Mean	1633734	**Mean**	1565779
Standard deviation	1567.32	**Standard Deviation**	750.6201
%RSD	**0.10%**	**%RSD**	**0.05%**

Inter day precision A standard solution containing (12, 24, 36µg/ml) of sacubitril (13, 26, 39 µg/ml) of valsartan were analyzed three times on a different day and % RSD was calculated. The results are given in table 11.

Table 11: Interday data for sacubitril and valsartan.

Sacubitril		Valsartan	
	Area		**Area**
Day 1	**1633733**	**Day1**	**1567013**
	1633499		**1565224**
	1632141		**1565091**
Day2	**1632778**	**Day2**	**1564452**
	1629361		**1567701**
	1634978		**1569969**
Mean	**1634978**	**Mean**	**1569969**
Standard deviation	**1915.63**	**Standard Deviation**	**2074.29**
%RSD	**0.12%**	**%RSD**	**0.13%**

Robustness

Small deliberate changes in chromatographic conditions such as a change in mobile phase ratio (±2%), change in wavelength (±2 units) and flow rate (±2 units) were studied to determine the robustness of the method. The results were in the factor of (% RSD<2%) the developed RP-HPLC method for the analysis of sacubitril and valsartan. The results are given in table 12.

Table 12: Robustness data for sacubitril and valsartan at different flow rate and wavelength.

Level	Sacubitril		Valsartan	
	Retention time	**Tailing factor**	**Retention time**	**Tailing factor**
Change in	Flow rate (ml/min)		Flow rate (ml/min)	
-1 (0.9ml)	7.482	1.12	6.214	1.14
0 (1.0ml)	6.703	1.12	5.567	1.14
+1 (1.1ml)	6.960	1.13	5.788	1.14
Changed in	Wavelength (nm)		Wavelength (nm)	
-2 (242nm)	7.023	1.12	5.834	1.14
0 (244nm)	6.970	1.13	5.788	1.14
+2 (246nm)	7.030	1.12	5.834	1.15

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were found to be 0.096µg/ml and 0.293µg/ml for sacubitril and 0.280µg/ml and 0.849µg/ml for valsartan estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and

precise as it can detected and quantify with very low concentration. The result is given in table 13.

Table 13: Data for LOD & LOQ for sacubitril and valsartan.

Sr.No.	Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Sacubitril	0.096	0.293
2	Valsartan	0.280	0.849

Method development

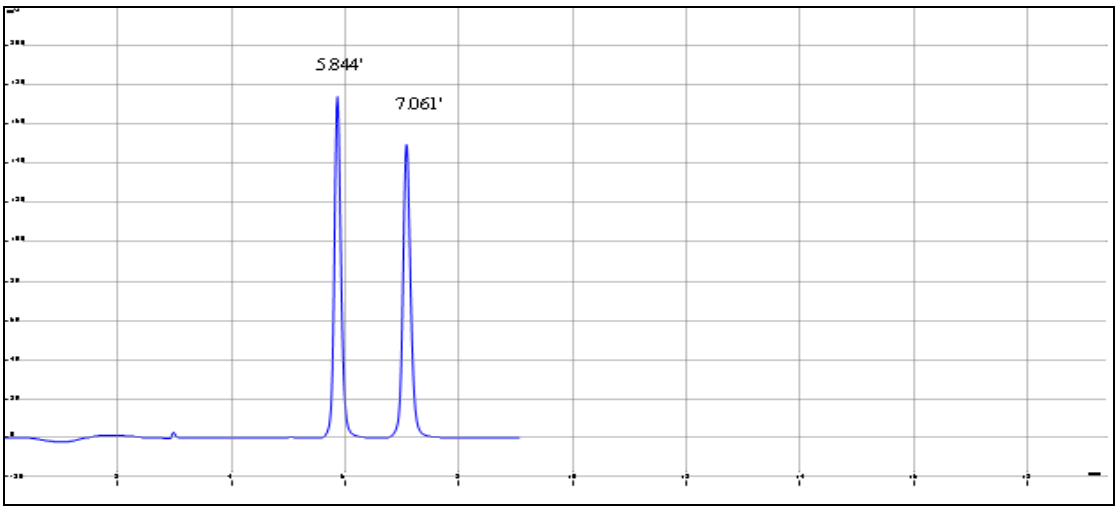
ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses were carried out.

Forced Degradation Study

Sacubitril and Valsartan were subjected to variety of stress conditions to affect degradation up to about 5-20%. The drugs were stressed under a variety of stress conditions like acid, alkali, effect by oxidation, light and dry heat. The stressed samples were subjected to chromatographic separation to resolve the drug from any potential degradation products.

Acid degradation

Acid decomposition studies were performed by refluxing 1 ml of sample stock solution was transferred into 10 ml of volumetric flask. 2 ml of 0.1N HCL was added and mixed well and put for 4 h at 70ºc in round bottom flask. After time period content was cooled to room temperature. Then the volume was adjusted with diluent to get 24ppm for sacubitril and 26ppm for valsartan. After making final solutions, it is injected into HPLC and peak area and peak shape were observed. Chromatogram of acid degradation on sample solution is shown in below fig 9.

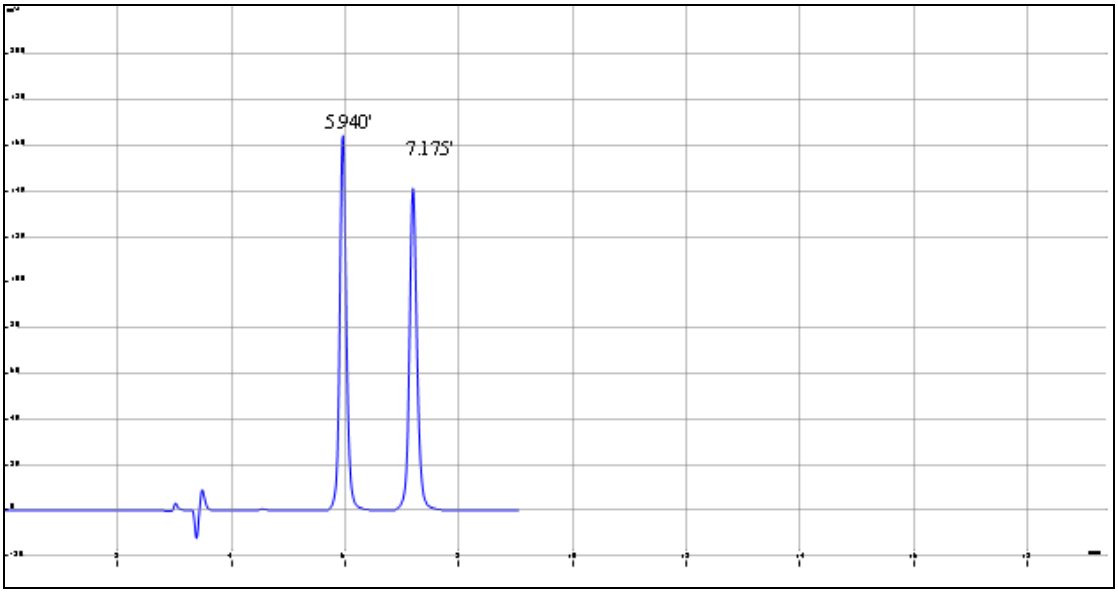


Time	Conc	Area	Resolution	T.Platenum	Asymmetry
5.844	26ppm	1945720	4.96	10652	1.14
7.061	24ppm	2575541	0.00	12144	1.12

Fig. 9: Acid degradation of sacubitril and valsartan at 4 hr.

Base degradation

Base decomposition studies were performed by refluxing 1ml of sample stock solution was transferred into 10ml of volumetric flask. 2ml of 0.1N NaOH solution were added and mixed well and put for 4 h at 70ºc in round bottom flask. After a time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 24ppm for sacubitril and 26ppm for valsartan. After making final solution, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of base degradation on sample solution is shown below fig 10.



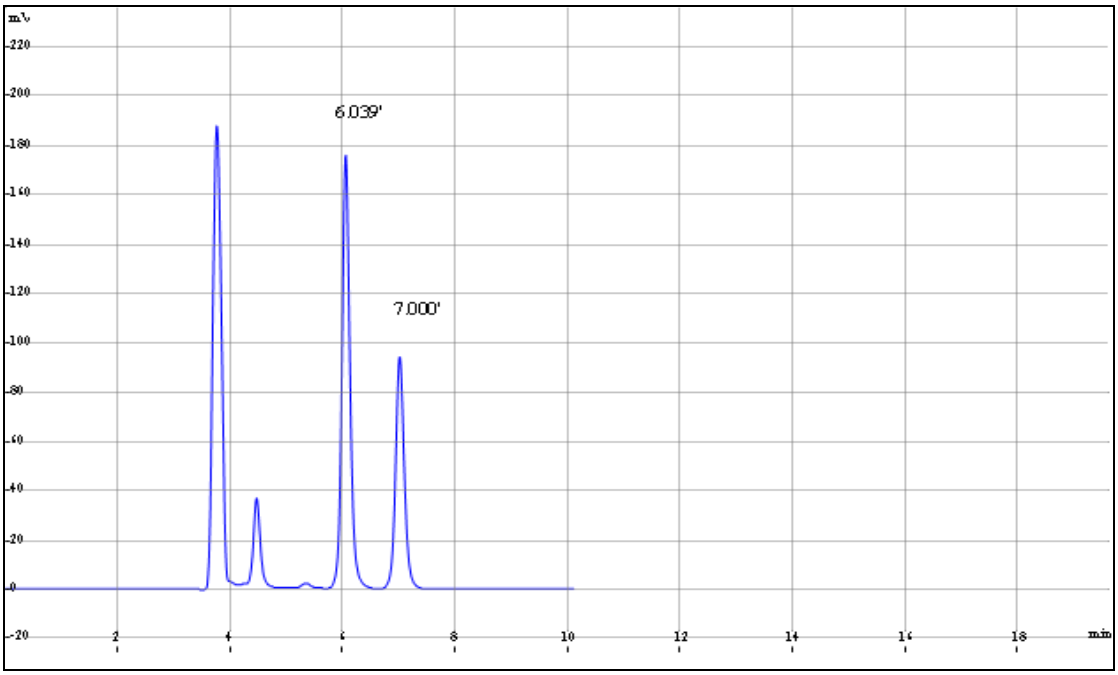
Time	Conc	Area	Resolution	T.Platenum	Asymmetry
5.940	26ppm	2015705	4.55	8024	1.12
7.175	24ppm	2389054	0.00	11343	1.10

Fig. 10: Base degradation of sacubitril and valsartan at 4 hr.

Oxidative degradation

Oxidative decomposition studies were performed by refluxing 1ml of sample stock solution was transferred into 10ml of volumetric flask. 2ml of 3% H_2O_2 solution were added and mixed well and put for 4 h at 70ºc in round bottom flask. After a time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 24ppm for sacubitril and 26ppm for valsartan. After making final solution, it is injected into HPLC and

the peak area and peak shapes were observed. Chromatogram of oxidative degradation on sample solution is shown below fig 11.

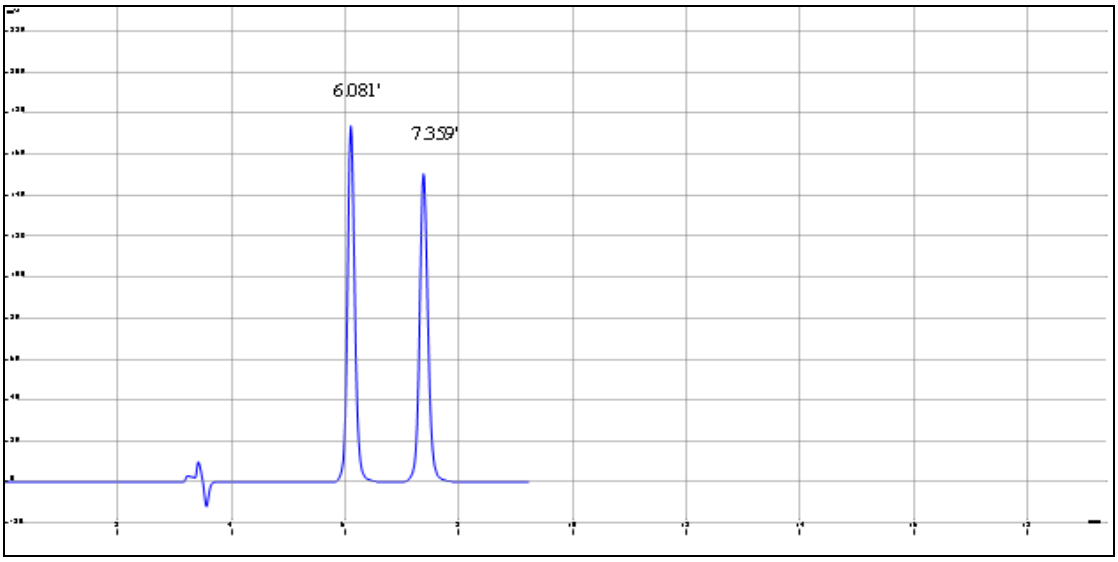


Time	Conc	Area	Resolution	T.Platenum	Asymmetry
6.039	26ppm	1920115	3.39	8297	1.13
7.000	24ppm	2061637	0.00	9161	1.10

Fig. 11: Oxidative degradation of sacubitril and valsartan at 4 hr.

Thermal degradation

Thermal decomposition studies were performed by taking 1ml of sample stock solution was transferred into 10ml of volumetric flask. The volumetric flask was stored in an oven at 110ºc for 4 h. Then the volume was adjusted with diluent to get 24ppm for sacubitril and 26ppm for valsartan. After making final solution, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of thermal degradation on sample solution is shown below fig 12.

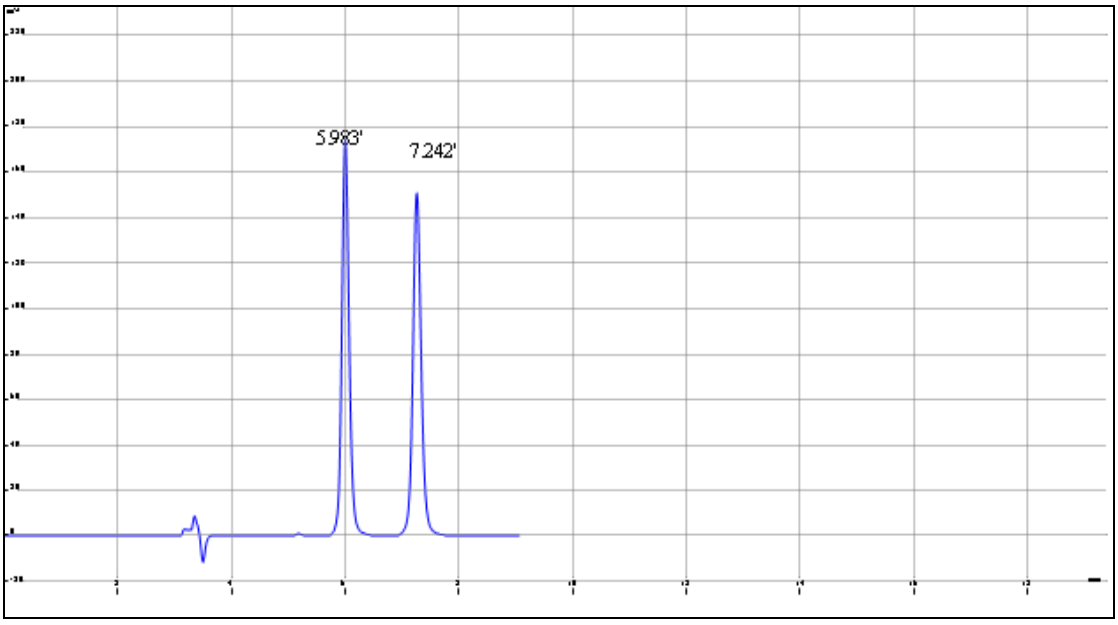


Time	Conc	Area	Resolution	T.Platenum	Asymmetry
6.081	26ppm	2642836	4.17	6728	1.12
7.359	24ppm	3009798	0.00	9170	1.10

Fig. 12: Thermal degradation of sacubitril and valsartan at 4 hr.

Photolytic degradation

Photolytic decomposition studies were performed by taking 1ml of sample stock solution was transferred into 10ml of volumetric flask. The volumetric flask was kept in an UV chamber for 1 72 h. Then the volume was adjusted with diluent to get 24ppm for sacubitril and 26ppm for valsartan. After making final solution, it is injected into HPLC and the peak area and peak shapes were observed. Chromatogram of photo degradation on sample solution is shown below fig 13.



Time	Conc	Area	Resolution	T.Platenum	Asymmetry
5.983	26ppm	2536176	4.15	6582	1.12
7.242	24ppm	2987341	0.00	9155	1.10

Fig. 13: Photolytic degradation of sacubitril and valsartan at 4 hr.

Table 13: Stability data for sacubitril.

Stress condition	Retention time	Area of peak	Degradation (%)	API after degradation %
Std. drug	6.984	13097587	--	--
Acidic (0.1N HCL)	7.061	2575541	80.33	19.67
Alkaline (0.1N NaOH)	7.175	2389054	81.75	18.75
Oxidation (H2O2)	7.000	2061637	84.25	15.75
UV	7.242	2987341	77.19	22.81
Thermal	7.359	3009798	77.02	22.98

Table 14: Stability data for valsartan.

Stress condition	Retention time	Area of peak	Degradation (%)	API after degradation %
Std. drug	5.311	9773330	--	--
Acidic (0.1N HCL)	5.844	1945720	80.09	19.91
Alkaline (0.1N NaOH)	5.940	2015705	79.37	20.63
Oxidation (H2O2)	6.039	1920115	80.35	19.65
UV	5.983	2536176	74.05	25.95
Thermal	6.081	2642836	72.95	27.05

CONCLUSION

Stability indicating RP-HPLC methods have been developed and validated for the determination of sacubitril and valsartan in tablet dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The degraded products are well resolved, indicating the method can also be useful for determination of degraded products. The proposed method is found to be simple, accurate, precise and robust. Hence, it can be used successfully for the routine analysis of sacubitril and valsartan in pharmaceutical dosage forms and for analysis of stability samples obtained during accelerated stability study.

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