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RP-UPLC Method for Simultaneous Estimation of Sacubitril and Valsartan in Its Bulk and Tablet Dosage Form with Force Degadation Studies

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Abstract : A simple, precise and accurate RP-UPLC method for simultaneous quantification of Sacubitril and Valsartan in bulk and tablet dosage form was developed and validated. Sacubitril and Valsartan were separated and estimated using waters UPLC with Inertsil ODS (1.7 x 150mm, 3 m size) column. The mobile phase used was phosphate buffer: acetonitrile (50:50%v/v). The elution of analytes was achieved with a flow rate of 0.4 ml/min and UV detection at a wavelength of 271 nm. The detector response was linear in the concentration range of 12-60 µg/mL and 13-65 µg/ml. The limits of detection, limit of quantification were 0.0626 µg/ml, 0.1897µg/ml and 0.0678µg/ml,0.2055µg/ml for sacubitril and valsartan respectively. The method was validated following ICH guidelines. All parameters of validation were found to be in the acceptance range.
Keywords : Sacubitril, Valsartan, Reversed-phase ultra-performance liquid, chromatography. Validation.

Introduction:

Sacubitril(SAC) is chemically (S)-5-[(4-phenylphenyl) methyl] pyrrolidine-2-one belongs to the class of neprilysin inhibitor, used as anti-hypertensive drug [13-14]. Its molecular Formula –$C_{17}H_{17}NO$ and molecular Weight – 251.32 g/mol. It is slightly soluble in water, sparingly in dehydrated alcohol and freely in methanol [20-23] (figure 1). Valsartan (VAL) is chemically (2S)-3-methyl-2-(N-{[2'-(2H-1, 2, 3, 4-tetrazole-5-yl) biphenyl-4-yl] methyl} pentanamido) butanoic acid[24, 25]. Valsartan is potent Angiotensin II receptor blocker. It is mainly used as anti-hypertensive drug. Valsartan is official in IP and USP [20-21]. The molecular Formula is $C_{24}H_{29}N_5O_3$,its molecular weight is 435.5 g/mol and Soluble in Acetonitrile, practically insoluble in water, methanol. (Figure 2). Literature survey revealed that few analytical methods are reported for analysis of both the drugs, either individually or in combination by using ultraviolet (UV) spectrophotometry, high-pressure liquid chromatography (HPLC) [1-12].Till date, there are no published reports about simultaneous estimation of sacubitril and valsartan by RP-UPLC in bulk drug and in pharmaceutical dosage forms. Hence, an attempt has been made to develop a new method for simultaneous estimation and validation of SAC and VAL by employing UPLC with photo diode array detector (PDA) [19, 20] in the presence of their stress degradants in accordance with the International Conference on Harmonization (ICH) guidelines [21].

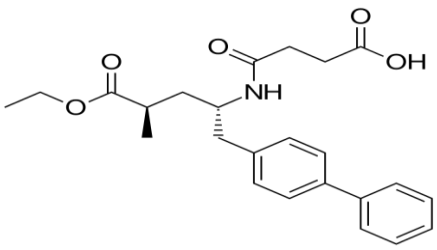


Figure1: Structure of Sacubtril

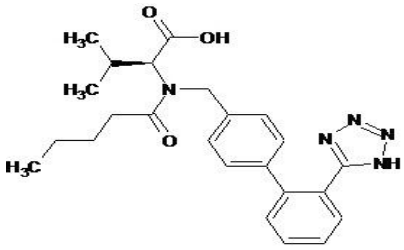


Figure2: Structure of Valsartan

Experimental:

Chemicals & Reagents:

All solvents were of HPLC grade and all reagents were of analytical grade ortho-phosphoric acid was obtained from merck (India). Acetonitrile was procured from MOLYCHEM. Water was purified with Milli-Q Plus, Millipore System (USA). All solvents and solutions were filtered through a membrane filter (Millipore Millex -HV filter units, 0.45 µm pore size; nylon) and degassed before use. All solutions were profiteered before injecting into HPLC system using Millipore milex hydrophilic PTFE unit filter of 0.45 µm pore size. Pharmaceutical grade of Sacubitril and Valsartan were procured from pharma train research solutions, Hyderabad.

Instrumentation:

The UPLC was carried out onwaters with empower 2695 separation module, auto Sampler and PDA Detector. Ultraviolet (UV)-visible spectrophotometer LABINDIA UV 3000^{+} with bandwidth of 10 mm matched quartz cell was used for all spectral measurements. Weighing was done on Afcoset ER-200A and P^{H} adjustments done using P^{H} meter Adwa – AD 1020 was used.

Chromatographic conditions:

Inertsil ODS (1.7 x 50 mm, 3µm) column was used in the study .The gradient separation was achieved using 500 ml of 0.1% ortho phosphoric acid Bufferand 500 ml of acetonitrile HPLC (50%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration. The flow rate of mobile phase was set as 0.4 ml per min .The column temperature was maintained at 25°C, and the detector was monitored at a wavelength 271 nm.

Equipment	**UPLC equipped with Auto Sampler and PDA Detector**
Column	Inertsil ODS (1.7 x 50 mm, 3µm)
Flow rate	0.4 mL per min
Wavelength	271 nm
Injection	5µl
Column oven	Ambient
Run time	6 min

Preparation of 0.1% ortho phosphoric acid Buffer: Pipetted 1 ml of ortho phosphoric acid in 1000 ml HPLC water.

Preparation of mobile phase: Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluent.

Preparation of stock solutions: Accurately weighed and transfered 12&13mg of Sacubitril & Valsartan working standard into a 10mL clean dry volumetric flask added diluent and sonicated to dissolve it completely

and made volume up to the mark with the same solvent. (Stock solution).Further pipetted out 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Furtherpipetted out 3.0ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution: Twenty tablets, each containing 24 mg of SAC and 26 mg of VAL, were weighed individually to determined the average weight and powdered separately in a mortar. Accurately weighed and Transfered equivalent to 12 &13mg of Sacubitril & Valsartan sample (Tablet powder) into a 10ml clean dry volumetric flask added about 7mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).Further pipetted out 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.Further pipetted out 3.0 ml of Sacubitril & Valsartanthe above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents.

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 UV spectrum wavelength selected as 271nm. At this wavelength, both the drugs show good absorbance. (Figure 3)

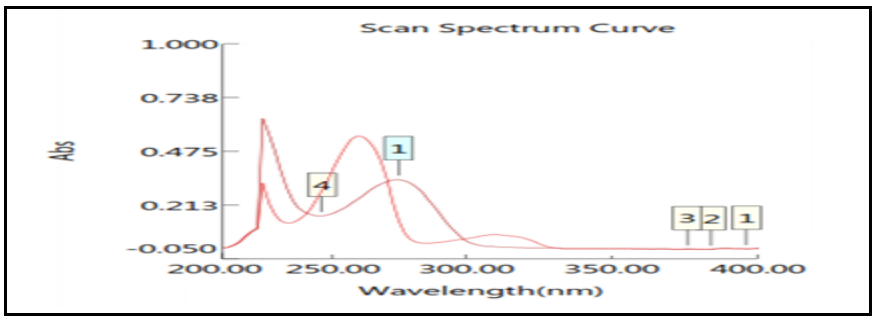


Figure 3: wavelengths of sacubtril and valsartan

Results and Discussion:

In order to achieve simultaneous elution of the two components, initial trails were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum P^H, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions, such as P^H3 phosphate buffer: Acetonitrile (60:40), P^H3 phosphate buffer: Acetonitrile (70:30), P^H4.5 phosphate buffer: Acetonitrile (50:50), 0.1%OPA: Acetonitrile (58:42), 0.1%OPA: Acetonitrile (40:60), 0.1%OPA: Acetonitrile (60:40).Finally, 0.1%OPA: Acetonitrile (60:40) in the ratio of 60:40 v/v was selected. After different flow variation trials 0.4 mL per min yielded optimum separation and peak shape. (Figure 4)

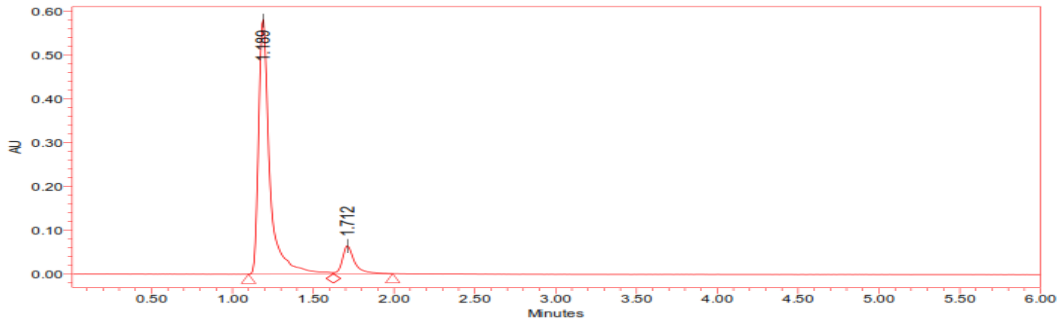


Figure4: Chromatogram of Sacubitril and Valsartan

Method validation

The developed method for the simultaneous estimation of sacubtril and valsartan was

Validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy,precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ) [18].

Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage of recoveries obtained sacubtril and valsartan was found to be 100.02 to 100.34% and 99.69-100.76% respectively.

Preparation of Accuracy solution: Accurately weighed and crushed by using mortar and pestle, transferred the sample equivalent to 50%,100%and 150% of sacubtril and valsartan working standard into a 100 ml clean dry volumetric flask and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock solution).Pipetted out 1.0 ml of sacubtril and valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.Further pipette out 3 ml of sacubtril and valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Table 1: Recovery study data of sacubtril and valsartan

SACUBTRIL							
Conc.	**Inj-1**	**Inj-2**	**Inj-3**	**Mean**	**% Recovery**	**SD**	**% RSD**
50%	528588	528167	522665	526473	100.02	3304.82	0.62
100%	1067099	1050006	1067921	1061675.33	101.04	10114.29	0.95
150%	1586385	1581376	1576679	1186148	100.34	4853.836	0.40
VALSARTAN							
50%	86649	87262	85214	86375	99.69	1051.13	1.21
100%	172298	176108	174890	174432	100.76	1945.85	1.11
150%	262091	260997	260920	261336	100.66	654.9817	0.25

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 103.0%

2. Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of method variability that can be expected for a given analyst performing the analysis and was determined by performing six replicate analysis of the same working solution [18]. The relative standard deviation (R.S.D.) obtained for sacubtril and valsartan are 0.37 and 0.46% respectively (Table 2).

Preparation of Precision solution: Accurately weighed and transferred 12&13mg of Sacubitril & Valsartan working standard into a 100ml clean dry volumetric flask, added diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Pipetted out 1.0 ml of 12&13mg of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipetted out 3 ml of 12&13mg of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Table 2: Summary of results of precision parameter for Sacubitril & Valsartan

SACUBITRIL						
	Inj-1	Inj-2	Avg	Mean	SD	% RSD
MP-1	1023945	1003903	1013924	1019980.58	5050.18	0.49
MP-2	1027796	1018214	1023005			
MP-3	1026845	1012117	1019481			
MP-4	1036375	1018518	1027447			
MP-5	1020865	1009168	1015017			

MP-6	1021653	1020368	1021011			
VALSARTAN						
MP-1	168040	164423	166232	165577.5	1108.51	0.66
MP-2	167914	165485	166700			
MP-3	160372	166719	163546			
MP-4	165848	165469	165659			
MP-5	166068	166045	166057			
MP-6	165321	165226	165274			

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

Linearity: Linearity was determined for Sacubitril in the range of 12-60 µg/ml and for Valsartan 13-65 µg/ml. The correlation coefficient ('r2') values for both the drugs were 0.999.

Preparation of stock solution: Accurately weighed and transferred 12&13mg of Sacubitril & Valsartan working standard into a 100ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Different concentration of sample ranging from 12 ppm to 60 ppm Sacubtril &13 ppm-65ppm Valsartan were prepared. (table 3)

Table 3:Summary of results of linearity parameter for Sacubitril and Valsartan

Sacubitril		**Valsartan**	
Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
12	360303	13	59045
24	692178	26	114337
36	1019720	39	168147
48	1343531	52	222495
60	1679118	65	276005
Correlation Coefficient	0.999		0.999

Limit of Quantitation (LOQ) and Limit of quantification (LOQ):

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels [18]. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.(table 4)

Table 4: Limit of Detection and Limit of Quantitation (LOQ) data for sacubtril and valsartan

Drug name	**Standard deviation(σ)**	**Slope(s)**	**LOD(µg/ml)**	**LOQ(µg/ml)**
Sacubitril	520041.1	27408	0.0626	0.1897
Valsartan	85711.31	4169	0.0678	0.2055

Robustness : The robustness of an analytical procedure is the measure of its ability to remain unaffected by small, but deliberate variations in method parameters. Robustness of the method was investigated by varying experimental conditions such as changes in flow rate and composition of mobile phase [18]. The mixed standard solution were injected every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained implies method is robust for routine quality analysis (Table 5).

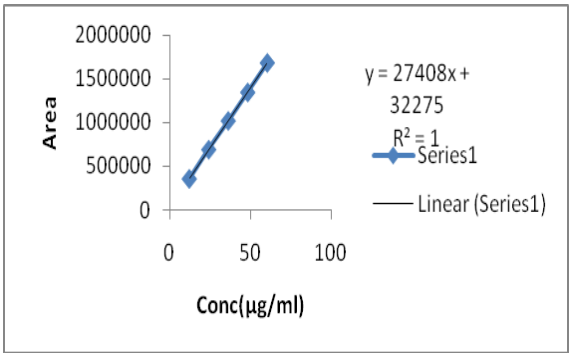


Figure 5: Linearity graph for Sacubtril

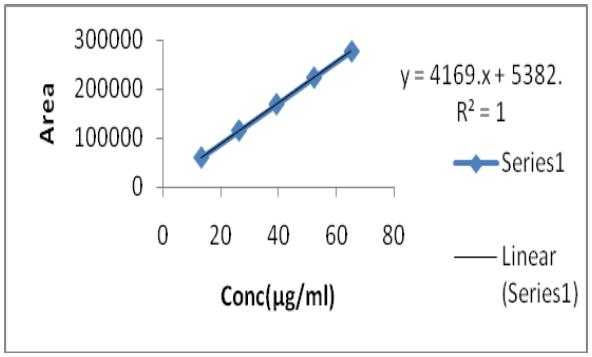


Figure 6: Linearity graph for Valsartan

Table 5: Summary of results of Robustness parameter for Sacubitril & Valsartan

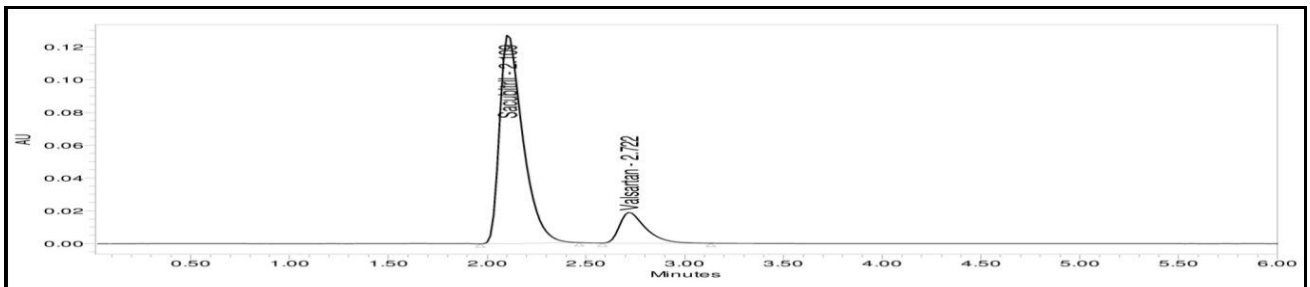
SACUBITRIL					
Flow Rate (ml/min)	**System Suitability Results**		**Change in Organic Composition in the Mobile Phase**	**System Suitability Results**	
	USP Plate Count	**USP Tailing**		**USP Plate Count**	**USP Tailing**
0.3	1680.7	1.6	10% less	1573.8	1.6
0.4	1524.84	1.6	*Actual	1524.84	1.6
0.5	1124.7	1.5	10% more	1124.7	1.5
VALSARTAN					
0.3	2200.8	1.7	10% less	2579.6	1.3
0.4	2177.99	1.4	*Actual	2177.99	1.4
0.5	1973.7	1.4	10% more	1973.7	1.4

Degradation studies:

ICH degradation was attempted to various stress conditions such as acid hydrolysis (using 0.1N HCl), base hydrolysis (using 0.1 N NaOH), oxidative hydrolysis (using 30% H2O2), thermal degradation (heated at 1100°C for 24hours) and photolytic degradation (overall illumination of ≥210Wh/m2 at 25°C for 7 days with UV radiation at 320-400 nm), to evaluate the ability of the proposed method to separate SAC and VAL from its degradation products. It was observed that SAC and VAL degrades with acidic, basic, oxidative and thermal stress conditions.The results are shown in table 6

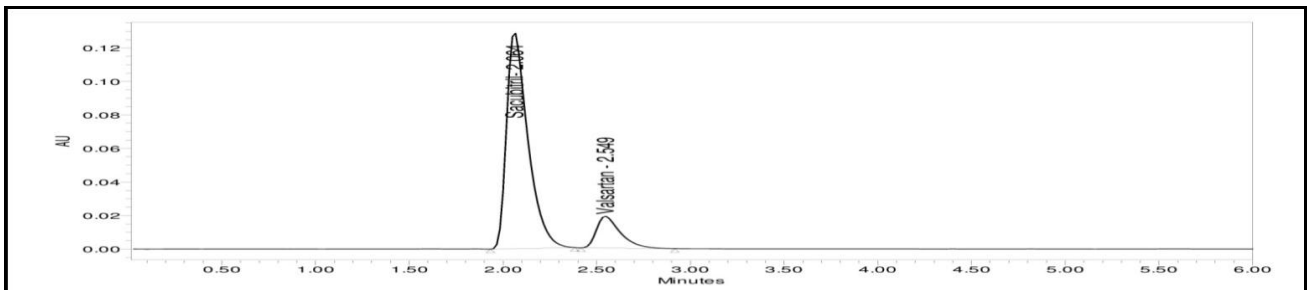
Table 6: Degradation data for sacubtril and valsartan

	Sacubitril	%degraded	Valsartan	%degraded
Standard	1050652		173068	
Acid	937570	10.8	151073	12.72
Base	920335	12.40	152390	11.94
Peroxide	1026845	2.26	152390	4.04
Thermal	1019720	2.94	168147	2.84
Photo	1018518	3.05	166045	4.05



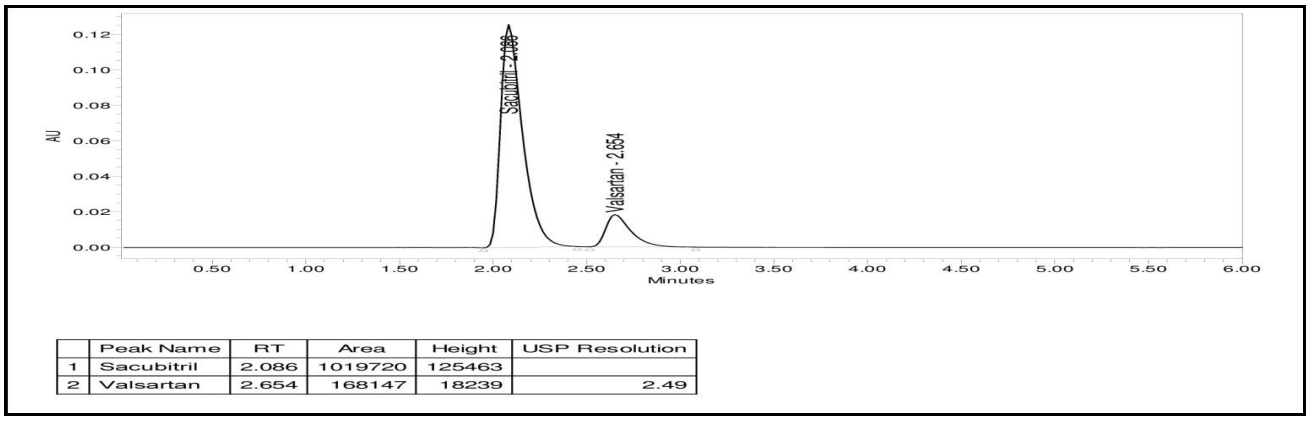
	Peak Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Sacubitril	2.109	937570	108521		1.59	2517.79
2	Valsartan	2.722	151037	15818	2.70	1.60	2104.65

Figure 7: Acid degradation chromatogram



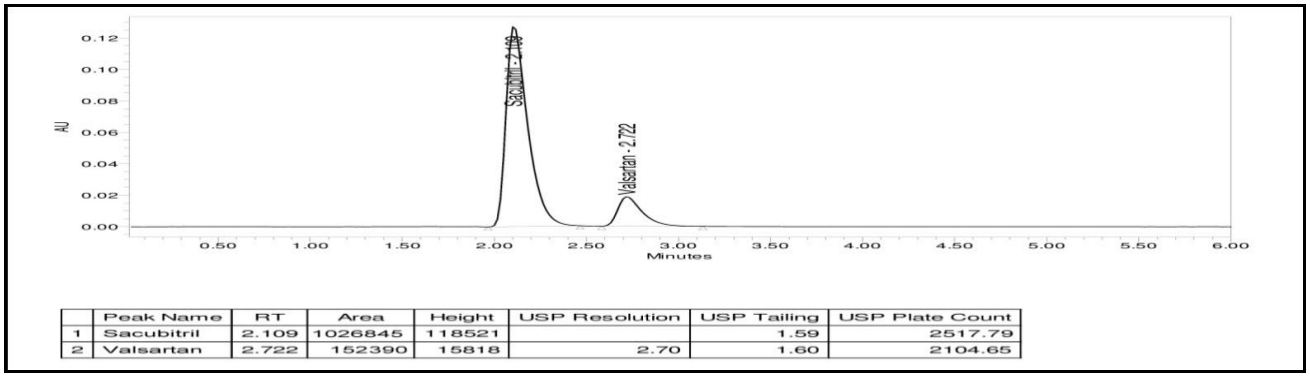
	Peak Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Sacubitril	2.064	920335	128604		1.54	2558.84
2	Valsartan	2.549	152390	18912	2.21	1.63	2978.12

Figure 8: Base degradation chromatogram



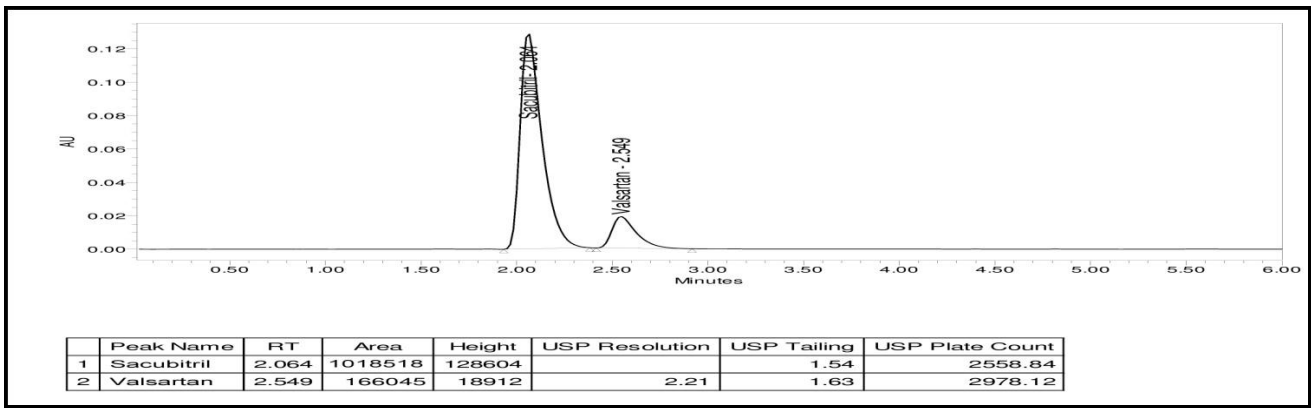
	Peak Name	RT	Area	Height	USP Resolution
1	Sacubitril	2.086	1019720	125463	
2	Valsartan	2.654	168147	18239	2.49

Figure 9: Thermal degradation chromatogram



	Peak Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Sacubitril	2.109	1026845	118521		1.59	2517.79
2	Valsartan	2.722	152390	15818	2.70	1.60	2104.65

Figure 10: Peroxide degradation chromatogram



	Peak Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Sacubitril	2.064	1018518	128604		1.54	2558.84
2	Valsartan	2.549	166045	18912	2.21	1.63	2978.12

Figure 11: Photolytic degradation chromatogram

Conclusion:

The present RP-UPLC method for simultaneous estimation of sacubtril and valsartan in their combined dosage form was established and validated as per the ICH guidelines. Linearity was achieved for sacubtril and valsartan in the range of 12-60µg/ml for sacubtril and 13-65µg/ml for valsartan with correlation coefficients (r2=0.999). The percentage recoveries of sacubtril and valsartan were achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. The forced degradation studies were performed byusing0.1 HCl, 0.1 NaOH, 3%H2O2, thermal, and photolytic degradation. Hence it can be used for the hyphenated instrumental analysis of sacubtril and valsartan in their bulk and combine dosage form.

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