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SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN PHARMACEUTICAL DOSAGE FORM IN DEVELOPMENT AND VALIDATION OF STABILITY INDICATING CHROMATOGRAPHIC METHOD

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Abstract:
The Present work was to develop and validate a stability indicating Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage form. The separation of Sacubitril and Valsartan was done using Inertsil ODS C_{18} column having dimension of (250 mm x 4.6 mm) having particle size of 5µm, with mobile phase consisting of Phosphate buffer (KH_2PO_4 and K_2HPO_4) pH 3 ±0.02 pH adjusted with ortho phosphoric acid,Methanol and Acetonitrile (30:50:20 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 241nm. The retention times of Sacubitril and Valsartan was found to be 2.927mins and 4.003mins. The proposed method has been validated for accuracy, precision, linearity, robustness, LOD and LOQ.Range were within the acceptance limit according to ICH guidelines. Linearity for Sacubitril and Valsartan was found in range of 50-150 µg/ml and correlation coefficient was found to be 0.997 and 0.997% RSD for precision for repeatability was 0.001672 and 0.002061,%mean recovery for Sacubitril and Valsartan was found to be 100.25% to 100.72% respectively. The results have showed that Sacubitril and Valsartan and the other degradation products were fully resolved and thus the method is stability-indicating.The developed method can be successfully employed for the routine analysis of Sacubitril and Valsartan in API and Pharmaceutical dosage forms.

Key Words: *Sacubitril,Valsartan, RP-HPLC, Method development, Validation.*

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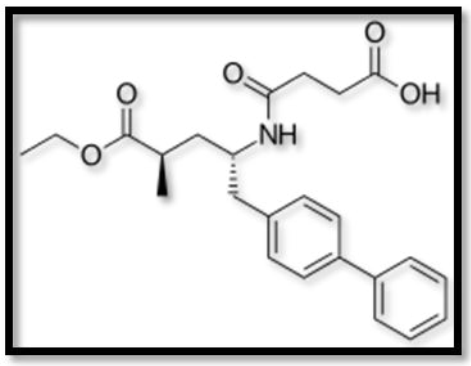
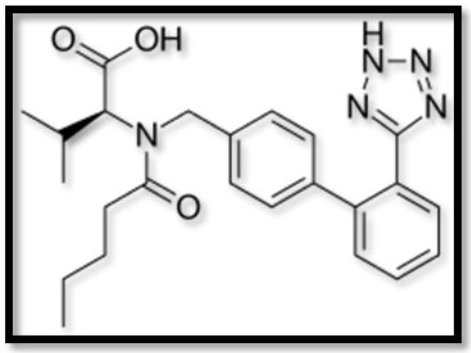
INTRODUCTION:

Valsartan is a nonpeptide, orally active and specific angiotensin II receptor blocker acting on the AT1 receptor subtype. Valsartan is chemically N-(1-oxopentyl)-N-[[2'-(1Htetrazol-5- yl)[1,1'-biphenyl] -4-yl]methyl]-Lvaline. [1-4] Methods such as HPLC[5-7], LC-MS[8-9] , protein precipitation[10] and simultaneous UV-spectrophotometric methods[11-12] are reported for estimation of valsartan alone or in combination with other agents. Sacubitril is chemically 4- [[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl)pentan-2-yl]amino]-4-oxobutanoic acid. [13] Sacubitril is an antihypertensive drug used in combination with valsartan for the treatment of heart failure.[14-15] Literature search reveals that only two analytical methods were reported for simultaneous estimation of sacubitril and valsartan from rat plasma using LC-MS/MS[16] and from a synthetic mixture using HPLC. [17] There is no stability indicating analytical methods were reported for simultaneous estimation of sacubitril and valsartan. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for the simultaneous estimation of sacubitril and valsartan from API and pharmaceutical dosage form.



Chemical structure of Sacubitril



Chemical structure of Valsartan

MATERIALS AND METHODS:

Materials and reagents Acetonitrile, potassium di-hydrogen phosphate, orthophosphoric acid and methanol of HPLC and AR grade were procured from Merck and Rankem lab ltd. Sacubitril and valsartan standards were received as gift samples from KP LABS.

Equipment :

Chromatographic separation was performed on HPLC system consist of model Separation module 2695 having UV detector 2487 and rheodyne injector with 20µl loop volume. LC Solution software was applied for data collecting and processing. UV spectrophotometer which consists of model U.V Win Software is also used to measure the wavelength of the solution of Sacubitril and Valsartan.

Preparation of Standard solution :

Weigh accurately 98mg of Sacubitril Working Reference Standard and 102mg Valsartan Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved in 10ml of mobile phase and make up volume with mobile phase. (Stock solution)From the above stock solution 98mcg/ml of Sacubitril and 102mcg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase.This solution is used as the working standard concentration for recording chromatogram.

A)Preparation of Sacubitril Standard Stock Solution (1000 µg/ml)

10mg of Sacubitril was weighed and transferred into 10ml of volumetric flask. Mix half diluent and shake it well.Then the volume was made upto the mark using the diluent.And prepare 10mcg/ml of solution by diluting 1ml to 10ml with methanol.

B)Preparation of Sacubitril Working Standard Solution (10µg/ml)

Pipette out 1ml of standard stock solution in other 10ml volumetric flask.Volume was made upto the mark with the diluent. Further dilute 1ml from the above solution in another 10ml volumetric flask.Then again the volume was made upto the mark using the diluent.

A)Preparation of Valsartan Standard Stock Solution (1000 µg/ml)

10mg of Valsartan was weighed and transferred into 10ml of volumetric flask. Mix half diluent and shake it well.Then the volume was made upto the mark using the diluent. And prepare 10mcg/ml of solution by diluting 1ml to 10ml with methanol.

B)Preparation of Valsartan Working Standard Solution (10 µg/ml)

Pipette out 1ml of standard stock solution in other 10ml volumetric flask.Volume was made upto the mark with the diluent. Further dilute 1ml from the above solution in another 10ml volumetric flask.Then again the volume was made upto the mark using the

diluent.

Preparation of Sample solution :

5 tablets were weighed & calculate the average weight of each tablet. Then the weight equivalent to 1 tablet was transferred into a 50 ml of volumetric flask. Add sufficient quantity of mobile phase & sonicated for 5 mins. After that filter the solution using 0.45-micron syringe filter and make up to 50 ml(100 µg/ml) with mobile phase. From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and make up to with mobile phase(10µg/ml).

Chromatographic conditions :

An Inertsil ODS C18 (250*4.6 mm, 5µm) column was used as the stationary phase. A mixture of phosphate buffer (pH 3.0) Orthophosphoric acid,Methanol and Acetonitrile in the ratio of (30:50:20 %v/v) was used as a mobile phase and pH 3.0 adjusted with ortho phosphoric acid. It was filtered through 0.45µ (micron) membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 241nm. The injection volumes of sample and standard were 20µl (microliter). Total run time is 6 mins.

RESULTS AND DISCUSSION:

Method Validation :

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System Suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factor was calculated. The results are given in table 1.

Table 1: System suitability parameters for sacubitril and valsartan

NAME	RT	USPTAILING	PLATE COUNT	RESOLUTION
SACUBITRIL	2.927	1.194	2779	
VALSARTAN	4.003	1.974	2548	3.620

Linearity:

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of sacubitril and valsartan at different concentration levels. The calibration curve showed (Fig.4 and 5) good linearity in the range of 50-150 µg/ml, for sacubitril with correlation coefficient (R^2) of 0.997 and 50-150 µg/ml for valsartan with correlation coefficient (R^2) of 0.997 Results are given in table 2.

Tab 2: Linearity for Sacubitril and Valsartan

SACUBITRIL			**VALSARTAN**	
S.No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	58.8	485.279	61.2	1331.154
2	78.4	697.105	81.2	1859.595
3	98	768.173	102	2010.885
4	117.6	1062.233	122.6	2728.440
5	137.2	1245.814	142.8	3196.923

Conc*- concentration

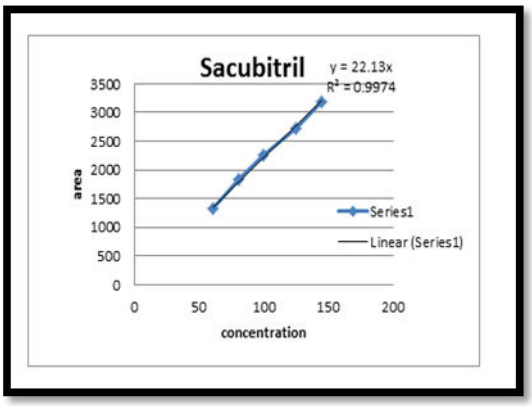


Fig4: Calibration curve of sacubitril

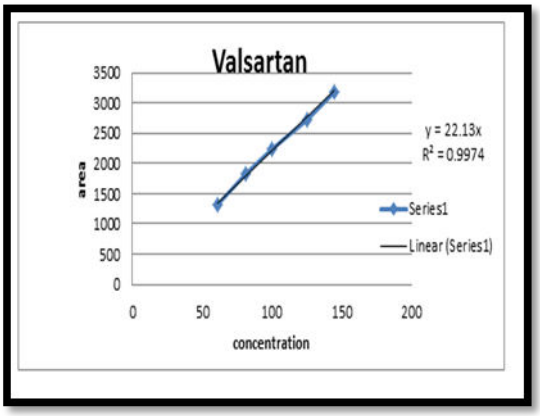


Fig 5: Calibration curve of valsartan

Accuracy : Recovery studies were carried out by addition of 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, 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 101.2% for the Sacubitril and 100.599% for valsartan at three different concentrations 80%, 100%, 120%. The results are given in table 3 and 4.

Table 3: Accuracy data for sacubitril

Recovery level	**Accuracy of Sacubitril**					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
80%	8.1	646754	648293.3	101.91	101.91	101.22%
	8.1	648998				
	8.1	649128				
100%	15	1172743	1174011.1	99.66	99.66	
	15	1174031				
	15	1175259				
120%	23.3	1866742	1868236.3	102.09	102.09	
	23.3	1867956				
	23.3	1870011				

S.D* - Standard deviation,Conc*- concentration Number of experiments (n) - 3

Table 4: Accuracy data for valsartan

Recovery Level	**Accuracy of Valsartan**					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
80%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
120%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

S.D* - Standard deviation Conc*- concentration Number of experiments (n) – 3

Precision Repeatability Standard solution containing sacubitril (10 µg/ml) and valsartan (10 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated. The results are given in table 5 and 6.

Table 5: Method precision data for sacubitril

S.No	**Injection**	**Peak Name**	**R_t**	**Area**
1	Injection-1	**Sacubitril**	**2.917**	**765.269**
2	Injection-2	**Sacubitril**	**2.92**	**760.464**
3	Injection-3	**Sacubitril**	**2.923**	**768.53**
4	Injection-4	**Sacubitril**	**2.923**	**763.825**
5	Injection-5	**Sacubitril**	**2.917**	**762.172**
6	Injection-6	**Sacubitril**	**2.93**	**775.496**
Average Area: 765.9593,**Avg Rt :** 2.921667				
STD of area: 5.42459,**STD of Rt:** 0.004885				
%RSD of area: 0.007082,**%RSD of Rt:** 0.001672				

S.D* - Standard deviation R.S.D* - Relative standard deviation Conc*- concentration Number of experiments (n) – 5

Table 6: Method precision data for valsartan

S.No	Injection	Peak Name	R_t	Area
1	Injection-1	Valsartan	3.993	2012.58
2	Injection-2	Valsartan	3.993	2009.765
3	Injection-3	Valsartan	3.99	2023.634
4	Injection-4	Valsartan	3.987	2028.946
5	Injection-5	Valsartan	3.99	2031.51
6	Injection-6	Valsartan	4.01	2035.765
Average Area:2023.7,**Avg Rt:**3.993833				
STD of area : 10.50642**, STD of Rt:** 0.008232				
%RSD of area:0.005192 **, %RSD of Rt:** 0.005192				

S.D* - Standard deviation R.S.D* - Relative standard deviation Conc*- concentration Number of experiments (n) – 5

ROBUSTNESS:

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Table 7: Robustness data for Sacubitril and valsartan

Parameter	SACUBITRIL		VALSARTAN	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.480	1.343	4.767	1.093
1.0 ml/min	2.933	1.194	4.030	1.974
1.2 ml/min	2.523	1.107	3.460	1.882
Wavelength				
239nm	2.920	1.267	3.993	1.921
241nm	2.940	1.300	4.050	1.000
243 nm	2.930	1.358	4.010	1.000

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

The LOD and LOQ were found to be 0.72µg/ml and 2.20µg/ml for sacubitril and 2.20µg/ml and 4.74µg/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 8.

Table 8 : LOD and LOQ of Sacubitril and Valsartan

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg/ml)	LOQ (µg/ml)
Sacubitril	369381	15579	0.72	2.20
Valsartan	618048	39092	2.20	4.74

Ruggedness

Ruggedness was evaluated by carrying out analysis of standard and sample solution containing (10mcg/ml of SAC and 10mcg/ml of VAL) on two different analysts using sample operational and environmental conditions.

Table 9: Ruggedness values of Sacubitril and Valsartan

	Sacubitril	Valsartan
Analyst 1	99.63	99.89
Analyst 2	99.33	98.65
%RSD	1.30%	1.24%

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

Acid degradation:

Acid degradation was performed by refluxing 5 ml of sample solution to it add 1 ml of 0.1 N HCL and sonicate. Place it aside for 3 hrs, then neutralize the solution with 1ml of base and then transfer above solution into 10 ml volumetric flask, dilute with mobile phase and record the chromatogram. Chromatogram of acid degradation on sample solution is shown below in figure 6.

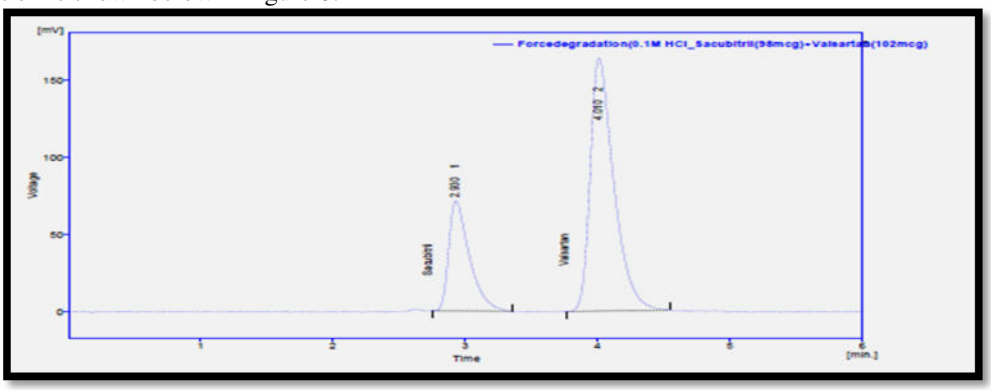


Fig 6:Chromatogram of Acid Degradation

Alkaline degradation

Alkaline degradation was performed by refluxing 5 ml of sample solution to it add 1 ml of 0.1 N NaOH and sonicate. Place it aside for 3 hrs, then neutralize the solution with 1ml of acid and then transfer above solution into 10 ml volumetric flask, dilute with mobile phase and record the chromatogram. Chromatogram of alkaline degradation on sample solution is shown below in figure 7

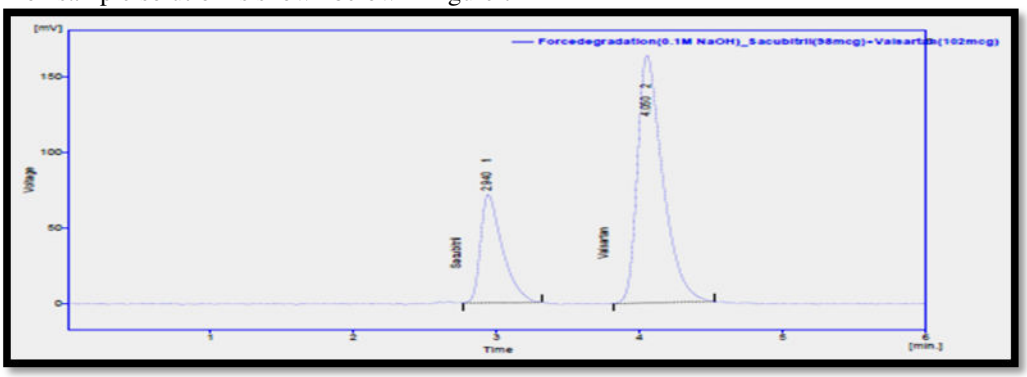


Fig 7 : Chromatogram of Alkaline Degradation

Oxidative degradation:

Oxidative Degradation was performed by refluxing 5 ml of sample solution to it add 1 ml of 3% H_20_2 and sonicate. Place it aside for 3 hrs, then transfer above solution into 10 ml volumetric flask, dilute with mobile phase and record the chromatogram**.** . Chromatogram of Oxidative degradation on sample solution is shown below in figure 8.

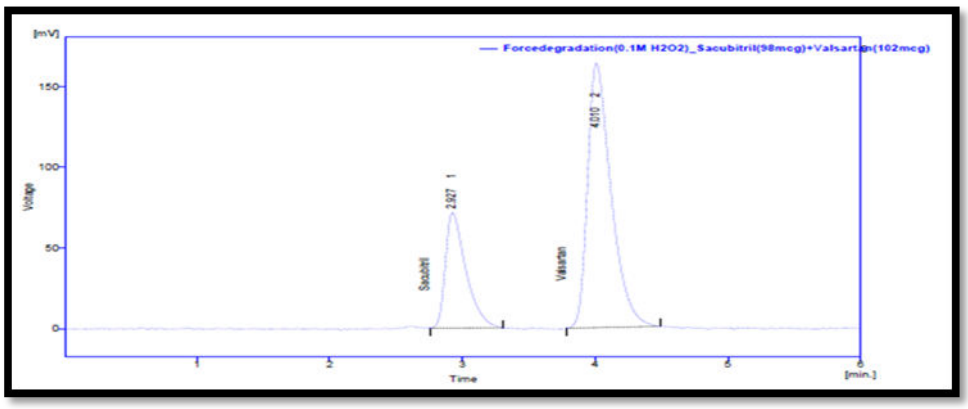


Fig 8: Chromatogram of Oxidative Degradation

Photolytic degradation:

Expose about 100 mg of sample in UV light chamber at 241 nm for 3 hrs. Weigh accurately this power equivalent to 98 mg of Sacubitril and 102 mg of Valsartan into a 100 ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram. Chromatogram of Photolytic degradation on sample solution is shown below in figure 9.

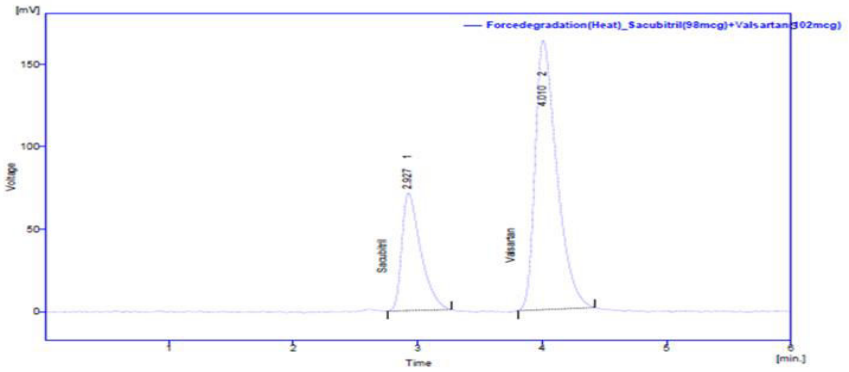


Fig 9 : Chromatogram of Photolytic Degradation

Thermal degradation:

Expose about 100 mg of sample in to dry heat at 80^0C for 3 hrs. Weigh accurately this powder equivalent to 98mg of Sacubitril and 102mg of Valsartan into a 100ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram. Chromatogram of Thermal degradation on sample solution is shown below in figure 10.

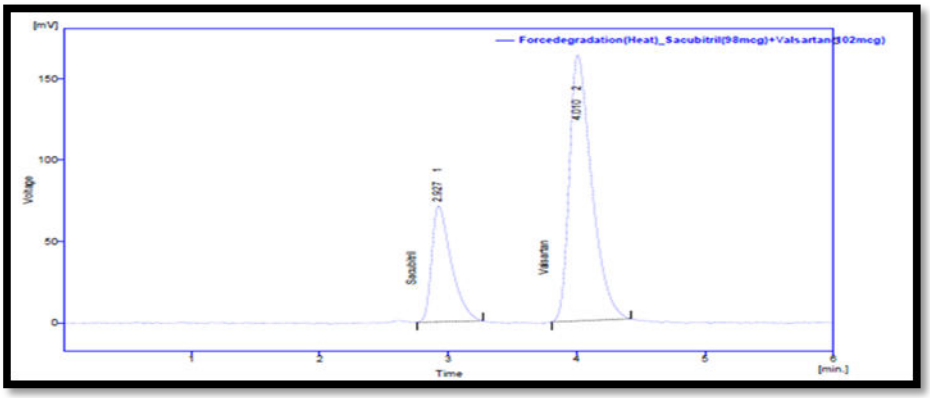


Fig 10 : Chromatogram of Thermal Degradation.

Table 10 : Stability data for sacubitril and valsartan

STRESS CONDITION	PEAK AREA		% DEGRADATION	
	SAC	VAL	SAC	VAL
Unstressed sample	772.321	2014.800	0	0
Acid	775.496	2035.765	0.41	1.040
Alkaline	760.494	2011.154	1.53	0.18
Oxidation	765.777	2010.401	0.84	0.21
Photolytic	766.992	2032.155	0.68	0.86
Thermal	757.879	1981.613	1.86	1.64

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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