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Advances in Bioresearch

ORIGINAL ARTICLE

Sacubitril and Valsartan Formulation Assay Method Development and Stability Study by RP-HPLC

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ABSTRACT
A stability indicating high performance liquid chromatographic (HPLC) method was developed and validated for the estimation of combined tablet formulation of valsartan and sacubitril. The HPLC system consisted of Shimadzu gradient HPLC with dual λ Absorbance UV detector. The wavelength of detection as set at 263nm. Separation was carried out in isocratic mode on Xterra C18 column (4.6x250mmx5µm) and the retention time of sacubitril and valsartan was found to be 1.54 min and 2.81 min respectively, using mobile phase consisting methanol, acetonitrile, and potassium dihyrogen phospahate, pH 3.6 in the ratio of 30: 60:10 v/v at a flow rate of 1ml/min with UV detection at 263nm. Good linearity obtained over the range of 20µg/ml to 160µg/ml for valsartan and sacubitril. Correlation coefficient was found to be 0.999&0.998 for sacubitril and valsartan respectively. The % RSD of precision for sacubitril and valsartan was found to be 0.31 and 0.27 respectively. The % mean recovery was found to be 99.20-99.54% for valsartan and 99.85-100.90% for sacubitril. The results obtained for accuracy, precision, LOD, LOQ and ruggedness were within the limits. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of valsartan and sacubitril in bulk and tablet dosage form. Thus, the validated economical method was applied for forced degradation study of valsartan and sacubitril tablet.
Keywords : Sacubitril, Valsartan, Recovery, Precision, Linearity.

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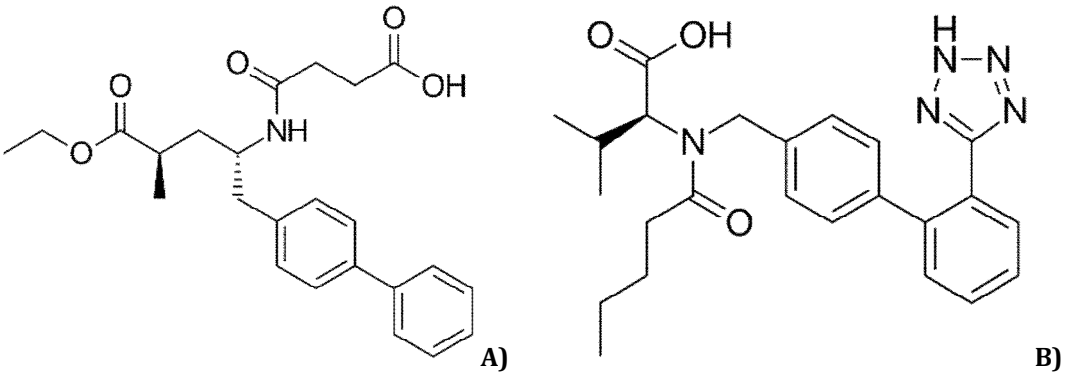
INTRODUCTION

Valsartan [1-3], chemically N-(1-oxopentyl)-N-[[2'-(1Htetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- L-valine [Figure 1A] is an angiotensin-receptor blocker (ARB) is used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Sacubitril [4-6] is chemically 4-[[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl) pentan-2-yl] amino]-4-oxobutanoic acid [Figure 1B], 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 [7-12].

Till date, only few analytical 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.



A) B)

Fig. 1: Chemical structures of A) Sacubitril and B) Valsartan.

MATERIAL AND METHODS

Chemicals and reagents

HPLC grade methanol, acetonitrile and analytical grade trifluro acetic acid were purchased from Merck (Mumbai, India). Sacubitril and valsartan standards were received as gift samples from Manus Akketeva and Lupin Ltd, India, respectively. Entresto tablets each containing 24mg of sacubitril and 26 mg of valsartan Manufactured by Novarties Pharmaceuticals Ltd. were obtained from local retail pharmacy

Instrumentation

The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. HPLC column BDS 250mm x 4.6 mm, 5µ. Mobile phase filtration unit (Pall Life sciences, Mumbai, India), LAB-INDIA U.V with UV Win software, Sonicator, P^H meter (LAB-INDIA), digital balance (Denver).

Preparation of pH 3.5, potassium dihydrogen phospahate

Dissolve 1.36 gm of potassium dihydrogen orthophosphare and 2ml of triethylamine in 800ml of water, adjust the pH to 3.5 with ortho phosphoric acid and add sufficient water to produce 1000ml.

Preparation of Calibration standard solutions

Stock solutions (1mg/ml) of valsartan and sacubitril were prepared in methanol. Further dilutions were carried out using 50% Methanol as diluent. Working standards of different concentrations ranging from 20µg/ml to 160µg/ml for valsartan and sacubitril were prepared by diluting several aliquots of standard solutions of valsartan and sacubitril.

Preparation of sample solution

Twenty tablets{Entresto) each containing 24mg of sacubitril and 26 mg of valsartan were weighed and powdered equivalent to dose, transferred to a 100 mL volumetric flask, and extracted with methanol. The mixture was sonicated for 20 min in an ultrasonic bath. The volume was adjusted to 100 mL with the same solvent and then filtered. Transfer 1ml of solution into a 10 ml volumetric flask and diluted up to the mark with diluents. to obtain Final concentration of sacubitril and valsartan was found to be 24 and 26µg/ml respectively.

Preparation of standard solution

Standard solution of concentration 24 µg/ml of sacubitril and 26 µg/ml of valsartan were prepared by dissolving exactly 24mg of sacubitril and 26 mg of valsartan drug product in a 100 ml clean volumetric flask containing diluents (60% methanol) and sonicated and made up to the mark and filtered through 4.5 µ filter under vacuum filtration. from this solution 1.0 mL was pipetted and the volume was made up to 10mL with diluents to get the concentration 24 µg/ml of sacubitril and 26 µg/ml of valsartan.

Assay procedure

Equilibrate the HPLC system and analyze the sample and standard solution as per optimized chromatographic conditions in triplicate (n=3) and the percent of assay was calculated from the peak area of standard and sample.

%Assay= (AT/AS)*(WS/WT)*(DT/DS)*(P/100)*(Average weight/Label claimed)*100.

Where: AT = Average area counts of test, AS = Average area counts of standard, WS = Weight of standard taken in mg, WT = Weight of sample taken in mg, DT = Dilution of test, DS = Dilution of standard, P = Percentage purity of working standard, LC = Label claim of drug mg/ml.

Stability studies

The objective of stability studies is to determine the percent of the sample found to be stable when it was subjected to different chemical and physical degradation conditions such as 0.1N HCl (acid hydrolysis), 0.1N NaOH (base hydrolysis), 3% H2O2 (oxidation), heat (thermal decomposition) and UV-light (radiation decomposition) for specified time.

Preparation of Degradation samples for Specificity Study

In order to establish whether the analytical method and the assay were stability-indicating, malarone tablets and pure active pharmaceutical ingredient (API) of both sacubitril and valsartan were stressed under various conditions to conduct forced degradation studies. As these drugs are freely soluble in methanol, it was used as a solvent and diluent in all the forced degradation studies.

RESULTS AND DISCUSSION

Method Development

The development of liquid chromatographic method was based on physico-chemical properties such as molecular weight, molecular formula, chemical structure, solubility, pKa value and UV absorption maxima of selected drugs. The selected drugs were completely soluble in water and methanol. Hence a reversed phase liquid chromatographic technique was adopted. The optimum chromatographic conditions were established by different trials by changing one of the chromatographic conditions such as column, mobile phase and its composition, flow rate of the mobile phase, injection volume, run time, column temperature and detection wavelength keeping other constant.

Optimized Chromatographic Conditions

Chromatographic Conditions the HPLC system consisted of Shimadzu gradient HPLC (JAPAN) with dual λ Absorbance UV detector. The wavelength of detection as set at 263nm. Separation was carried out in isocratic mode on Xterra C18 column (4.6x250mmx5µm) and the retention time of sacubitril and valsartan was found to be 1.54 min and 2.81 min respectively. (Figure 2), using mobile phase consisting methanol, acetonitrile, and potassium dihyrogen phospahate, pH 3.6 in the ratio of 30: 60:10 v/v at a flow rate of 1ml/min with UV detection at 263nm. The mobile phase filtered through nylon milli pore (0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultra sonicator prior to use. Chromatography was carried out at room temperature 25°C and maintains the column temperature at 32°C [Fig. 2].

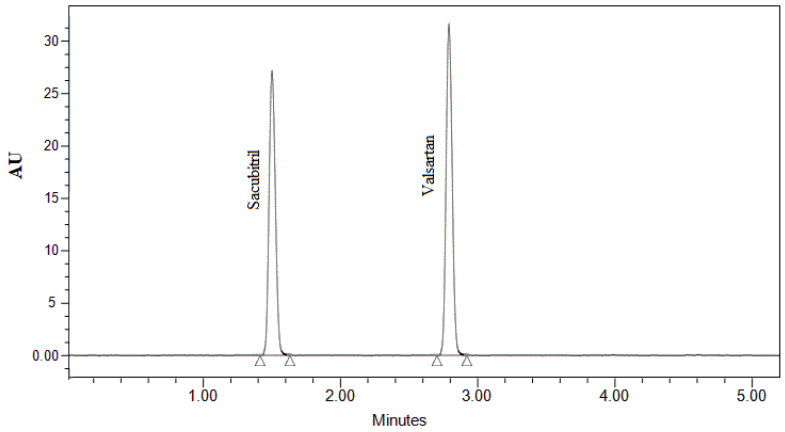


Fig. 2: Optimized Chromatogram of sacubitril and valsartan

The developed Method was validated for linearity, precision, accuracy, ruggedness and is applied for forced degradation studies as per the ICH guidelines.

Method validation [13-16]

Linearity

Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined by solutions containing 20µg/ml to 160µg/ml (Table 1) for valsartan and sacubitril [17]. Correlation coefficient was found to be 0.999 and 0.998 for sacubitril and valsartan respectively (shown in Fig. 3 and 4).

Table 1: Linearity data for valsartan& sacubitril

S.no	Sacubitril		Valsartan	
	Concentration(µg/ml)	Peak area	Concentration(µg/ml)	Peak area
1	20	5284	20	6041
2	40	9728	40	10734
3	60	14260	60	15849
4	80	19689	80	21347
5	100	24135	100	25761
6	120	29541	120	31474
7	140	34581	140	35250
8	160	39517	160	41502

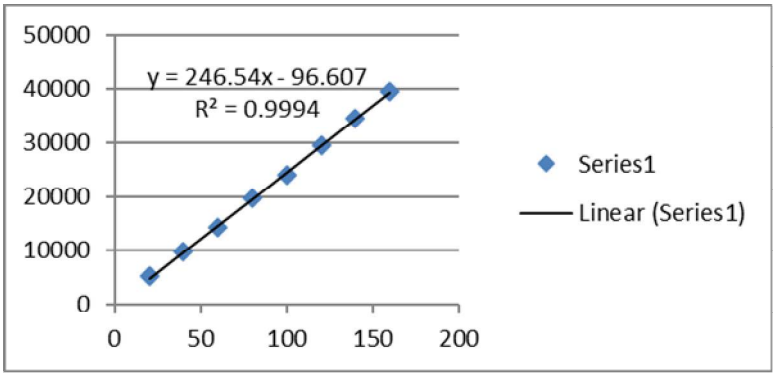


Fig. 3: Calibration curve of sacubitril

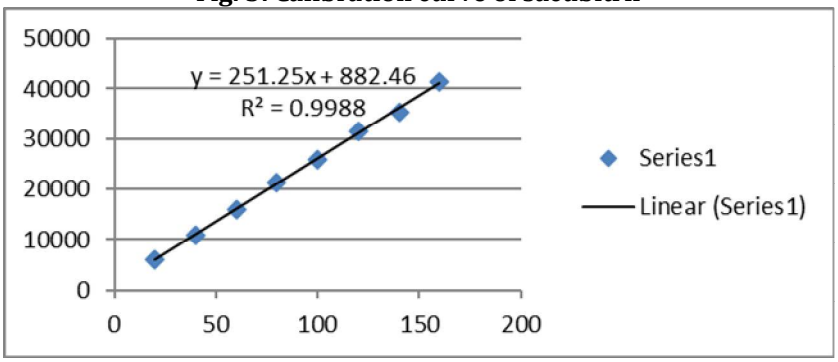


Fig. 4: Calibration curve of valsartan

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is calculated using the formula 3.3 times σ/s where "σ" is standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve[18]. Similarly LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ are shown in Table 2.

Table 2: Linearity, LOD and LOQ findings of sacubitril and valsartan

Sacubitril		Valsartan	
Conc (µg/ml)	**Avg Area**	**Conc (µg/ml)**	**Avg Area**
20	5284	20	6041
40	9728	40	10734
60	14260	60	15849
80	19689	80	21347
100	24135	100	25761
120	29541	120	31474
140	34581	140	35250
160	39517	160	41502
Intercept	-96.6		882.4
slope	246.5		251.2
Intercept Standard Deviation	115.36		213.69
LOD (µg/ml)	1.54		2.80
LOQ(µg/ml)	4.68		8.50

Precision

The intraday precision was demonstrated by injecting standard solutions of valsartan and sacubitril with 40µg/ml and 140µg/ml respectively as per the test procedure[19]. The % RSD of Sacubitril and Valsartan was found to be 0.31 and 0.27respectively (Fig. 5 and Table 3).

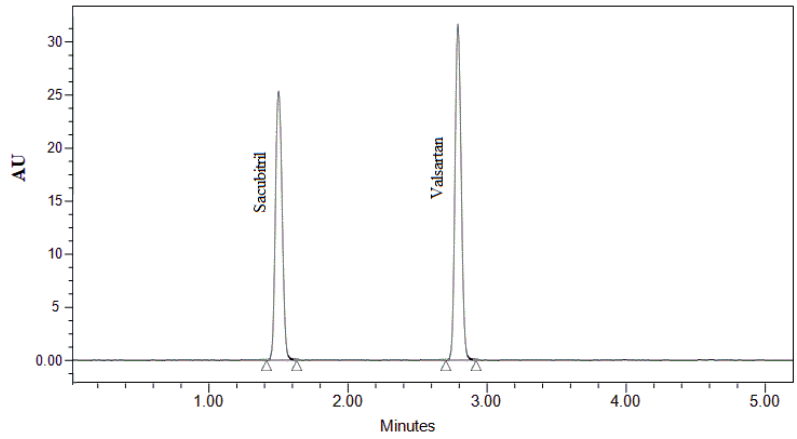


Fig. 5: Chromatogram showing standard injection.

Table 3: Method Precision data of Valsartan and Sacubitril

Sacubitril (40µg/ml)		Valsartan (140µg/ml)
S.No	**Area**	**Area**
1	9826	34987
2	9741	35016
3	9798	35126
4	9814	35195
5	9804	35203
6	9818	35183
Mean	9800	35118
SD	30.64	94.87
%RSD	0.31	0.27

Intermediate Precision

Intermediate precision of the analytical method was determined by performing method precision on in three successive days by different analysts under same experimental condition by injecting six replicate standards preparations was determined and the mean % RSD of sacubitril (40µg/ml) and valsartan (140µg/ml) was found to be 0.32 and 0.29 respectively (Table 4)[20].

Table 4: Precision Data for sacubitril & valsartan

	Sacubitril Area for 40µg/ml				Valsartan Area for 140µg/ml			
S.No	**day-1**	**day-2**	**day-3**	**avg**	**day-1**	**day-2**	**day-3**	**avg**
1	9806	9797	9787	9797	34917	34882	34847	34882
2	9722	9712	9702	9712	34946	34911	34876	34911
3	9778	9769	9759	9769	35056	35021	34985	35021
4	9794	9785	9775	9785	35125	35089	35054	35089
5	9784	9775	9765	9775	35133	35097	35062	35097
6	9798	9789	9779	9789	35113	35077	35042	35077
Mean	9781	9771	9761	9771	35048	35013	34978	35013
SD	30.6	30.6	30.5	30.6	94.69	94.59	94.5	94.59
%RSD	0.31	0.31	0.31	**0.32**	0.27	0.27	0.27	**0.29**

Accuracy

Accuracy of the method was established by performing recovery studies according to the ICH guidelines. Spiked samples were prepared by spiking pre-analyzed sample solutions with pure drug at three different concentration levels each in triplicate (Fig. 6 to 8). Mean percentage recovery values at three different concentrations of the two drugs was calculated. The % mean recovery of sacubitril (99.20-

99.54%) & valsartan (99.85-100.90.%) at each level was within the limits of 98% and 102% (Table 5)[21].

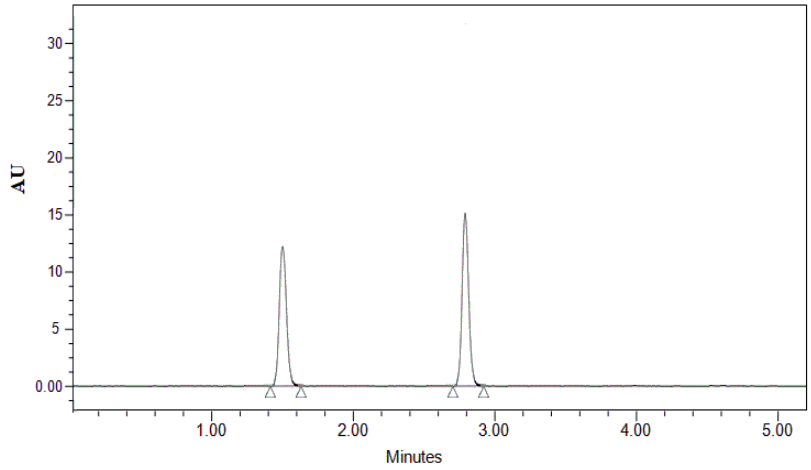


Fig. 6: Chromatogram showing accuracy 50%injection

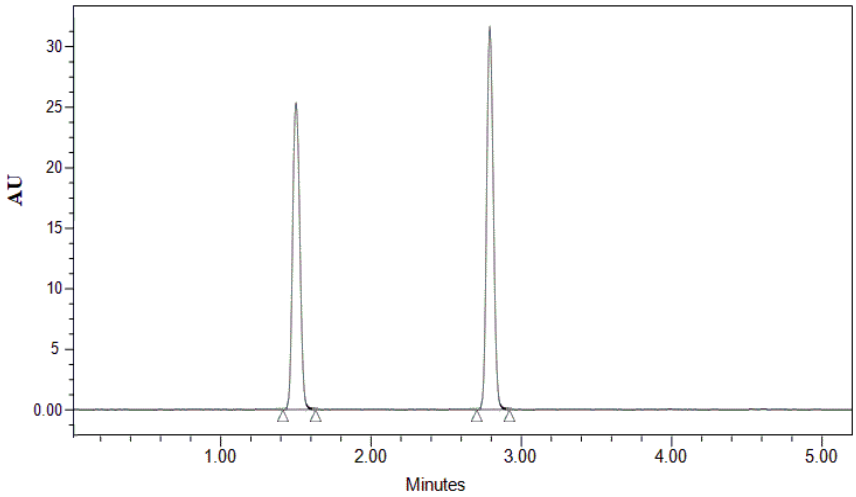


Fig. 7: Chromatogram showing accuracy 100%injection

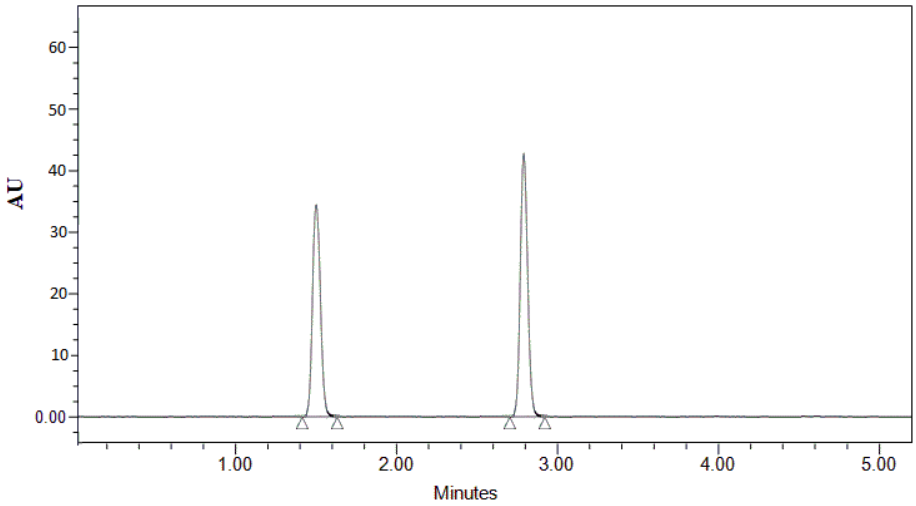


Fig. 8: Chromatogram showing accuracy 150%injection

Table 5: Accuracy of valsartan and sacubitril

Accuracy of Sacubitril						
S.N0.	**Conc.**	**Calculated Concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	80	79.54	99.43			
2		79.43	99.29	99.43	0.14	0.15
3		79.66	99.58			
1	160	159.38	99.61			
2		158.96	99.35	99.54	0.16	0.17
3		159.44	99.65			
1	240	240.05	100.02			
2		236.88	98.70	99.20	0.71	0.72
3		237.35	98.89			
Accuracy of valsartan						
S.N0.	**Conc.**	**Calculated concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	80	80.45	100.56			
2		80.55	100.69	100.90	0.48	0.48
3		81.16	101.45			
1	160	160.86	100.53			
2		160.98	100.61	100.57	0.038	0.04
3		160.92	100.57			
1	240	239.83	99.92			
2		239.51	99.79	99.85	0.069	0.07
3		239.59	99.83			

Ruggedness

The ruggedness of method for Valsartan and Sacubitril was calculated with six injections of 68µg/ml in two batches using two different columns[18-20]. The % CV of ruggedness for sacubitril was 0.14 with column-1 and 0.04 with column-2 and the % CV of ruggedness for valsartan was 0.04 with column-1 and 0.03 with column-2 (Table 6), which is within acceptance limits.

Table 6: Results of Ruggedness

	Sacubitril 160µg/ml		**Valsartan 160µg/ml**	
S.NO	**Column 1**	**Column 2**	**Column 1**	**Column 2**
1	159.06	159.12	159.18	159.14
2	159.06	159.2	159.21	159.01
3	159.34	159.09	159.14	159.04
4	159.54	159.22	159.02	159.02
5	159.15	159.11	159.15	159.09
6	159.55	159.24	159.04	159.11
Mean	159.28	159.16	159.12	159.06
± SD	0.22	0.06	0.07	0.052
% CV	0.14	0.040	0.048	0.033
% Accuracy	99.55	99.47	99.45	99.41

Results of Assay Studies

The mean percent of assay Entresto tablet (containing 24mg of sacubitril and 26 mg of valsartan) was determined by comparing the peak area of standard and formulation and found to be 99.2 for sacubitril and 99.1 for valsartan, and the results were given in Table 7.

Table 7: Results of assay studies of sacubitril and valsartan

S. No.	**Standard (Entresto)**		**Sample**		**% Assay**	
	Sacubitril 24 µg/ml	**Valsartan 26µg/ml**	**Sacubitril 24 µg/ml**	**Valsartan 26µg/ml**	**Sacubitril**	**Valsartan**
1	6447	7851	6332	7937	98.2	101.1
2	6238	7796	6194	7664	99.3	98.3
3	6337	7913	6350	7763	100.2	98.1
Mean	6341	7853	6292	7788	99.2	99.1

Results of Stress Degradation Studies

Stress degradation studies were performed as per the ICH guidelinesQ1A (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method [13,14].(Table 8)

Acid Degradation studies

To 1ml of stock solution valsartan and sacubitril, 1ml of 2N HCl was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 9)

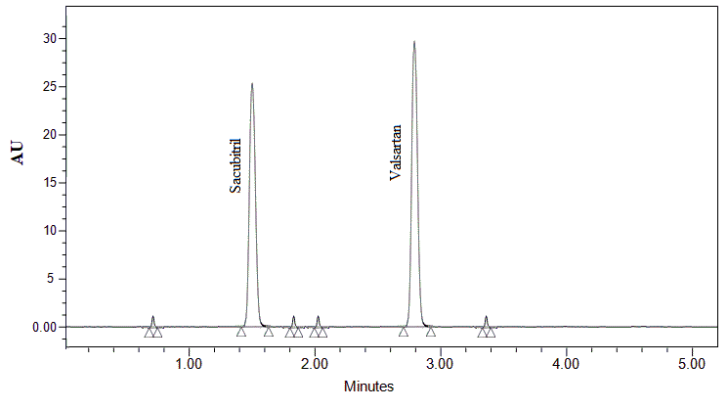


Fig. 9: Chromatogram of Acid Degradation

Alkali Degradation Studies

To 1ml of stock solution of standard drug and sample valsartan and sacubitril, 1ml of 2N NaOH was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 10)

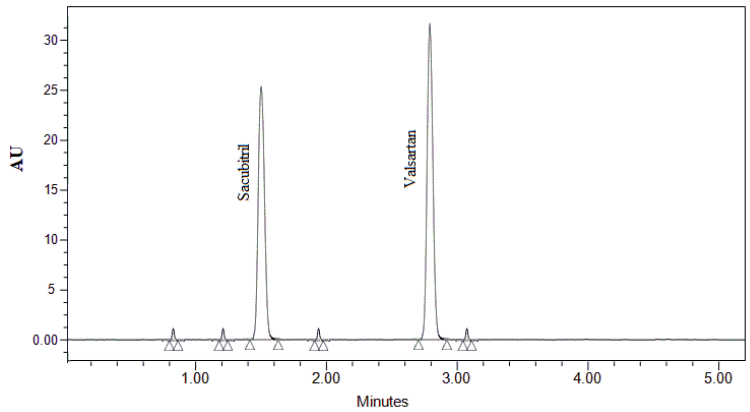


Fig.10: Chromatogram of Base Degradation

Oxidative Degradation

To 1ml of stock solution of standard drug and sample of valsartanand sacubitril, 1ml of 20% $H_2O_2$was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 11)

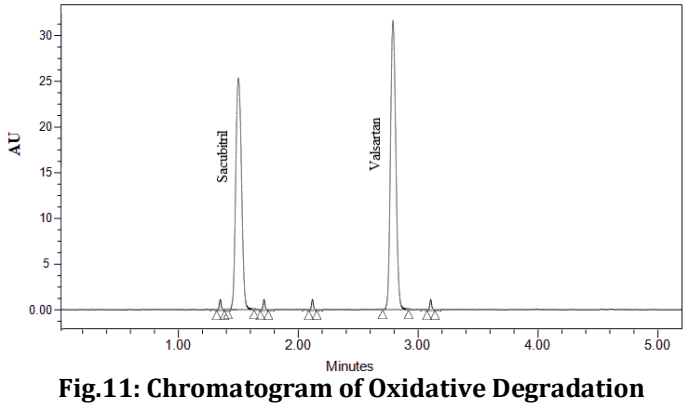


Fig.11: Chromatogram of Oxidative Degradation

Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the 36 µg/ml standard solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 12)

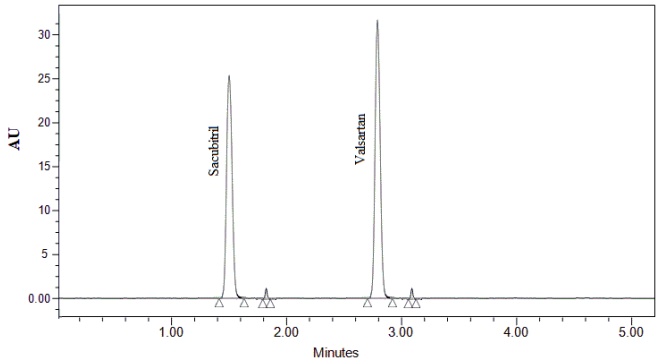


Fig.12: Chromatogram of UV Degradation

Thermal degradation studies

The 1ml of stock solution of standard drug and sample of valsartan and sacubitril was exposed to temperature 105°C for 24hrs for HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample.(Figure 13)

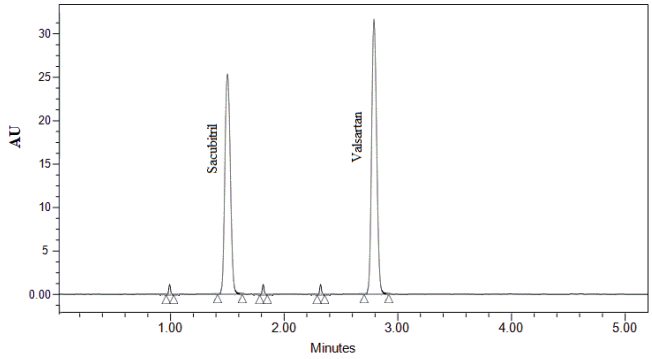


Fig. 13: Chromatogram of Thermal Degradation Study

Table 8: Results of stress degradation studies of sacubitril and valsartan

			Sacubitril		Valsartan	
Sno	**Stress conditions**	**Time**	**% Assay**	**% Degradation**	**% Assay**	**% Degradation**
1	Acid Degradation	30 min	91.2	8.8	97.3	2.7
2	Base Degradation	30 min	92.6	7.4	97.1	2.9
3	Peroxide Degradation	30 min	97.5	2.5	91.7	8.3
4	UV Degradation	7 days	96.6	3.4	95.8	4.2
5	Thermal Degradation	24hrs	97.8	2.2	98.4	1.6

CONCLUSION

The developed stability indicating HPLC-UV method for simultaneous estimation of valsartan and sacubitril was novel, simple, precise, accurate, robust & cost-effective method. In present study valsartan and sacubitril simultaneously estimated by HPLC, good linearity obtained for both drugs (20µg/ml-160µg/ml) with Correlation coefficient of 0.999&0.998 for sacubitril and valsartan respectively. The results for precision, recovery and ruggedness were within the limits. Hence the method was successfully applied for degradation studies, both the drugs undergoes significant degradation in acidic, oxidation, alkaline, and UV. Comparatively More degradation was found with acid and base for sacubitril and with peroxide for valsartan. As per ICH guidelines peak purity angle should be less than peak purity threshold. Hence, method of the analysis of valsartan and sacubitril in tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. hence the proposed analytical method is also useful for the determination of valsartan and sacubitril stability in sample of pharmaceutical dosage form.

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