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A New Way of Method Establishment and Validation of Related Substance of Sacubitril and Valsartan by RP-HPLC and its Forced Degradation Study was characterized by LCMS

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Abstract:
A novel, simple and accurate high performance liquid chromatographic method has been development with quantitative analysis of Sacubitril and Valsartan using Inertsil C_{18} 150x4.6mm, 3.5µ column with a flow rate of 1ml/min. The buffer containing 2.5g of Hexane sulphonic acid and 1ml of Triethyl amine of pH 2.5 adjusted with OPA and the mixture of two components like Buffer and Acetonitrile in the ratio of 70: 30 is used as mobile phase. The detection was carried out at 224nm. The proposed method shows good linearity in the concentration range from 2.6µg/ml to 36µg/ml for Sacubitril. Valsartan concentration range from 2.6µg/ml to 39µg/ml. Precision and recovery study results are in between 98-102%. In entire robustness conditions % RSD is below 2.0%. Degradation has minimum effect in stress condition and solutions are stable for 24hrs. Method validation is carried out according to ICH guidelines and the parameters are precision, accuracy, specificity, stability, robustness, linearity, limit of detection and limit of quantification are evaluated and the values are found to be within the acceptable limit. Finally the degradation products are characterized by using LC-MS technique.

Key words: ICH Guide lines, LCMS, RP-HPLC, Sacubitril and Valsartan.

1. INTRODUCTION

Sacubitril

Sacubitril drug is antihypertensive [1, 2] and it is used in combination drug Sacubitril and Valsartan [3, 4] their formulation marketly available by the name of Entresto this used used in treatment of heart failure [5, 6]. This drug was approved as FDA's [7] for the review process priority in use of heart failure on July 7, 2015. Sacubitril is a prodrug [8, 9] is activated to Sacubitrilat (LBQ657) by de-ethylation via eserases. Sacubitrilat inhibits the enzyme neprilysin [10, 11] which is accountable for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides [12] mainly it works reducing blood volume [13, 14]. In addition, neprilysin degrades a variety of peptides including bradykinin [15, 16], an inflammatory mediator exerting potent vasodilatory [17] action. Structures of Sacubitril and their impurities 1 and 2 shown in Fig. no's 1, 2 and 3.

Valsartan

Valsartan available in market by the name Diovan among others, it is used as medication to treat heart failure, high blood pressure [18], and diabetic kidney disease [19]. It is a cable for initial treatment for high blood pressure. Diovan directly taken by mouth. Different types of combination of Valsartan/hydrochlorothiazide and Valsartan/amlodipine [20]. Valsartan is an angiotensin receptor blocker (ARB) that may be used to manage hypertension and heart failure. Many studies have demonstrated the efficacy of Valsartan in reducing blood pressure (BP) in many patient population. Structures of Valsartan and their impurities A and B are shown in Fig. no's 4, 5 and 6.

By the literature search there is no article published so far for the references. The purposed method was simple and economical sensitive for the estimation of Sacubitril and Valsartan.

2. MATERIALS AND METHODS

2.1 Materials:

Acetonitrile, Ortho Phosphoric Acid (OPA), Hexane Sulphonic acid and Tri ethyl amine, water were purchased from Merck (India) Ltd. Worli, Mumbai, India. All API's of Sacubitril and Valsartan as reference standards were procured from Glenmark pharmaceuticals, Mumbai.

2.2 Equipments:

HPLC, make: Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

2.3 Chromatographic Conditions:

An instrument of HPLC system (Waters Alliance e2695 model), connected with LCMS (QTRAP 5500 triple quadrupole) was used to develop the method and its validation. Empower 2.0 software was used to processing the data. The column was Inertsil C_{18} 150x4.6mm, 3.5µ dimentions. The two compounds and their related impurities are separated by isocratic elution. Mobile phase having pH 2.5 buffer solution, Acetonitrile in the ratio of 70:30. Flow rate of pump was set as 1.0ml/min. The UV detection was captured at 224nm. Injection volume fixed as 10µl and the diluent was same as the mobile phase.

Preparation of Mobile Phase:

Preparation of Buffer: 2.5g of Hexane sulphonic acid and 1ml of Triethyl amine is added to water and adjust its pH to 2.5 with Ortho Phosphoric Acid.

Preparation of Mobile Phase: Buffer: Acetonitrile (70:30)

Diluent: Mobile Phase is used as diluent.

2.4 Preparation of Solutions

2.4.1 Preparation of Standard solution:

Weigh accurately 24mg of Sacubitril and 26mg of Valsartan standards are transferred into a 100ml

volumetric flask, then add 70ml of mobile phase and sonicate for 10min. to dissolve the contents make upto the mark with diluent. Further diluted 5ml of above solution to 50ml with diluent.

2.4.2 Prepartion of Sample solution:

Weigh accurately the weight equivalent to 50mg of equivalent weight of Azmarda tab formulation transferred into the 100ml volumetric flask, then add 70ml of diluent and sonicate to 10min to dissolve the contents completely and after that make upto the mark with diluent.

2.4.3 Preparation of Impurity standard stock solution:

Weigh accurately each 5mg of Sacubitril and Valsartan impurities into a 100ml volumetric flask. Add 70ml of diluent, sonicated to dissolve and make up to the mark with diluents.

2.4.4 Preparation of Spiked sample solution:

Transfer 5ml of sample solution into a 50ml volumetric flask, then add 30ml of diluent and also add 5ml of impurity standard stock solution and makeup to the mark with diluent. Filter through 0.45µ syringe filter.

2.5 Wavelength optimization:

The absorption spectra of solution of each Sacubitril and Valsartan two drugs are scanned over the range of 200-400 nm by using PDA detector and the spectra were recorded. By observing the spectrum we can found that impurities, Sacubitril and Valsartan showing maximum absorbance at 224nm. Hence, 224nm is selected for method validation.

2.6 Method Validation

The analytical method was validated as per ICH Q2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), forced degradation and stability.

2.6.1 System Suitability

System suitability parameters were measured to verify the system performance. The parametrers including USP plate count, USP tailing and % RSD are found to be within the limits.

2.6.2 Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

2.6.3 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excipients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Sacubitril and Valsartan.

2.6.4 Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

2.6.5 Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slople, intercept and correlation coefficient.

2.6.6 LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined base on the calibration curves. The LOD and LOQ for Sacubitril and Valsartan were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ were calculated as 3.3 s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

2.6.7 Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q_1A (R2). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

2.6.8 Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2ml/min), wavelength (±5nm), variation in pH (±0.2), organic content in the mobile phase (±10%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

2.6.9 Stability

Analytical solution was prepared and injecting into the HPLC system at periodic intervals of 0 hours to 24 hours at 6hour intervals depending on the instrument utilization and sequence of injection.

3. RESULTS AND DISCUSSION

Optimization of Method and Sample concentration

For the first chromatographic conditions selected for method is reversed-phase HPLC with Inertsil C_{18} 150x4.6mm, 3.5µ column with isocratic elution. Mobile phase is mixture of buffer and acetonitrile (70:30). The

flow rate is 1.0ml/min and the column temperature is ambient.

All impurity peaks are well separated with greater than 2 resolution. And there is no interference peaks was observed at Sacubitril and Valsartan with their impurities due to the blank and other excipients which are used in the tablet formulation. The spiked sample chromatogram was shown in the figure.7.

The parameters of the developed and validated HPLC method are presented in table 1. Recovery data and peak sharpness depends to finalized the diluent and sample concentration and injection volumes was finalized greater threshold than the limit of quantification (LOQ). The isocratic was optimized to get the best resolution. The optimized chromatographic conditions shown in table 1.

System suitability

The standard solution was introduced into HPLC system and found that system suitability parameters are within the limits. The percentage of RSD was calculated standard peak areas. The similar injections RSD percentage was observed and it is within the limit. The obtained results were presented in table 2 and the system suitability chromatogram was exhibited in the figure 8.

Specificity

A study was conducted to establish the placebo interference. As per the test method, samples are prepared equivalent weight of API and placebo with test concentration and then injected into HPLC system. Interference was not found for the chromatograms of placebo solution, empty cell solution and impurities solution at the retention time of Sacubitril and Valsartan and its impurities.

The typical chromatograms of specificity were shown in the figures 9, 10, 11 and 12. Interference was not found for the chromatograms of placebo solution, blank solution and impurities solution at the retention time of Sacubitril, Valsartan and its impurities.

Linearity

Sacubitril linearity concentration was prepared in the range of 2.4µg/ml to 36µg/ml for Sacubitril. The regression equation was found to be Y= 306220x+230176 and correlation coefficient is 0.999. Impurity-1 concentration range from 0.5µg/ml to 7.5µg/ml, regression equation is Y= 100068x+1214.5 and correlation coefficient was found to be 0.999. Impurity-2 concentration range from 0.5µg/ml to 7.5µg/ml, regression equation is Y= 121278x+7428.1 and correlation coefficient was obtained 0.999.

Valsartan linearity concentration range was prepared in the range of 2.6µg/ml to 39µg/ml, regression equation is Y= 337378x+15344 and correlation coefficient was found to be 0.999. Impurity-A concentration range from 0.5µg/ml to 7.5µg/ml regression equation is Y= 88750x+2772 and correlation coefficient was obtained 0.999. Impurity-B concentration range from 0.5µg/ml to 7.5µg/ml, regression equation is Y= 96279x+3092 and correlation coefficient was found to be 0.999. The linearity plots are shown in figures 13, 14,15,16,17 and 18.

Robustness

In Robustness there is a small deviation in flow rate (±0.2ml) and organic solvent (±20%) in their chromatographic condition there is no significant change in RSD (%). The obtained results were presented in table 3

Stability

Stability of Sacubitril and valsartan was determined in sample solution was studying initial to 24hr at different time intervals at room temperature and 2-8°C. There is no significant deviation of purity. The obtained results were listed in table 4.

Precision

Precision of the method was established by injecting test preparation and tested through the complete analytical procedure from sample preparation to the final result. Repeatability assessed using a minimum of 6 determinations and calculated % relative standard deviation of impurities. The obtained results are tabulate in table 5, 6.

Intermediate Precision

Six replicates of a sample solution were analysed on a different day, different analyst and different instrument. Peak areas were calculated which were used to calculate mean, % RSD values. The obtained results were presented in table 7, 8.

Limit of Detection and Quantification (LOD & LOQ)

LOD and LOQ were determined by calibration curve method. LOD and LOQ of the compound were determined by injecting progressively lower concentrations of standard solutions using developed RP-HPLC method. The slope method was used for estimation of LOD and LOQ and the equation used are LOQ= 10xσ/S and LOD= 3.3xσ/S, where S is the calibration curve slope and σ is the standard deviation of the response. The LOD and LOQ concentrations for Sacubitril is 0.02µg/ml and 0.24µg/ml and LOD and LOQ concentrations of Valsartan is 0.02µg/ml and 0.26µg/ml. The typical chromatogram of LOD and LOQ were shown in figures 19 and 20.

Accuracy

The accuracy of the related substances test procedure was determined by spiking of Sacubitril and Valsartan impurities stock solution to test the sample. So that the concentration of the impurity would be 5.0% of the test concentration as per the test method. Injecting samples in triplicate at 50%, 100% and 150% of the target concentration. The recovery results should be NLT 95% and NMT 105%. The obtained results were presented in table 9, 10, 11, 12, 13 and 14 and chromatograms were shown in the figures 21, 22 and 23.

Table 1: Optimized HPLC method conditions

S.No	Parameter	Method conditions
1	Column	Inertsil C_{18} 150x4.6mm, 3.5µ
2	Flow rate	1.0ml/min
3	Wave length	224nm
4	Injection volume	10µl
5	Run time	17min
6	Mobile phase	HSA+ACN (70:30)

Table 2: System suitability data for Sacubitril and Valsartan

S.No	System suitability parameter	Acceptance criteria	Drug Name	
			Sacubitril	Valsartan
1	% RSD	NMT 2.0	0.98	1.02
2	USP Tailing	NMT 2.0	0.51	0.89
3	USP Plate count	NLT 3000	5489	6541

Table 3: Robustness data

S.No	Parameter name	% RSD for purity	
		Sacubitril	Valsartan
1	Flow (0.8ml/min)	0.89	1.54
2	Flow (1.2ml/min)	0.54	0.48
3	Organic solvent (+10%)	1.10	1.25
4	Organic solvent (-10%)	1.09	1.37

Table 4: Solution stability results

S.No	Stability	Purity of Sacubitril in RT	Purity of Sacubitril in 2-8°C	Purity of Valsartan in RT	Purity of Valsartan in 2-8°C
1	Initial	100.18	100.11	99.98	99.96
2	6Hr	100.12	100.08	99.95	99.93
3	12Hr	100.05	100.01	99.91	99.87
4	18Hr	99.98	99.96	98.89	98.78
5	24Hr	99.84	99.78	98.34	98.29

Table 5: Precision results for Sacubitril

Sample No.	% of Related Substances		
	Spiked Impurities	Total Impurities	% Purity (100-Total Imp)
1	5.01	4.89	95.11
2	5.02	4.92	95.08
3	5.05	4.97	95.03
4	5.04	4.96	95.04
5	5.02	4.98	95.05
6	5.03	4.95	95.05
Average	5.03	4.95	95.06
% RSD	0.29	0.69	0.03

Table 6: Precision results for Valsartan

Sample No.	% of Related Substances		
	Spiked Impurities	Total Impurities	% Purity (100-Total Imp)
1	5.00	4.98	95.02
2	5.01	4.95	95.05
3	5.02	4.94	95.06
4	5.00	4.90	95.10
5	5.02	4.92	95.08
6	4.98	4.91	95.09
Average	5.01	4.93	95.07
%RSD	0.30	0.60	0.03

Table 7: Intermediate results for Sacubitril

Sample No.	% of related substances		
	Spiked Impurities	Total Impurities	% Purity (100-Total Imp)
1	5.00	4.98	95.02
2	5.01	4.95	95.05
3	4.98	4.94	95.06
4	4.99	4.99	95.01
5	5.02	5.00	95.00
6	5.01	4.98	95.02
Average	5.00	4.97	95.03
% RSD	0.29	0.47	0.03

Table 8: Intermediate results for Valsartan

Sample No.	% of related substances		
	Spiked Impurities	Total Impurities	% Purity (100-Total Imp)
1	5.00	4.97	95.03
2	4.98	4.92	95.08
3	5.01	4.95	95.05
4	5.02	4.89	95.11
5	4.97	4.84	95.16
6	5.02	4.98	95.02
Average	5.00	4.93	95.08
% RSD	0.42	1.08	0.06

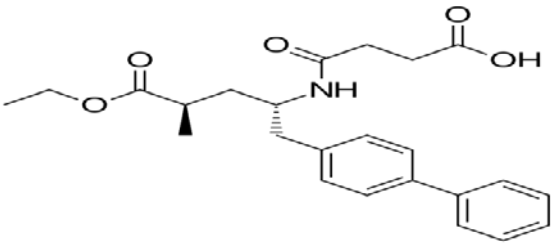

Fig. 1: Structure of Sacubitril

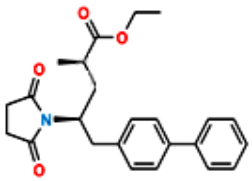

Fig. 2: Structure of Sacubitril Imp-1

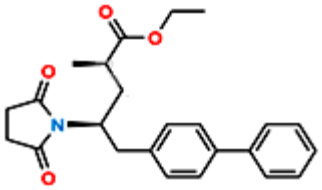

Fig. 3: Structure of Sacubitril Imp-2

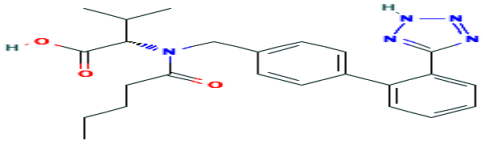

Fig. 4: Structure of Valsartan

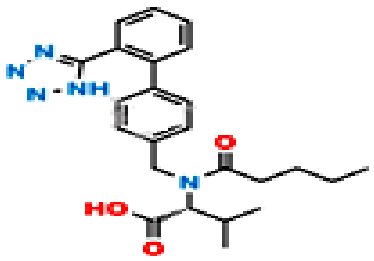

Fig. 5: Structure of Valsartan Imp-A

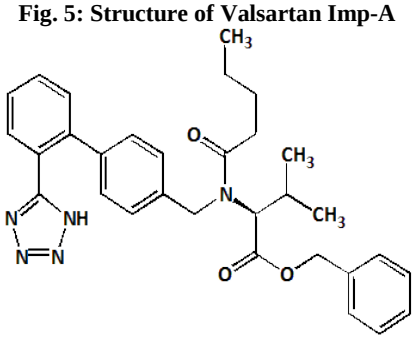

Fig. 6: Structure of Valsartan Imp-B

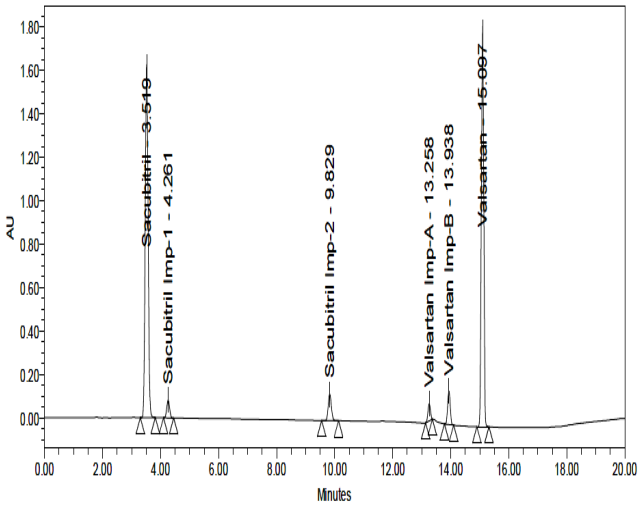

Fig. 7: Typical chromatogram of Standard

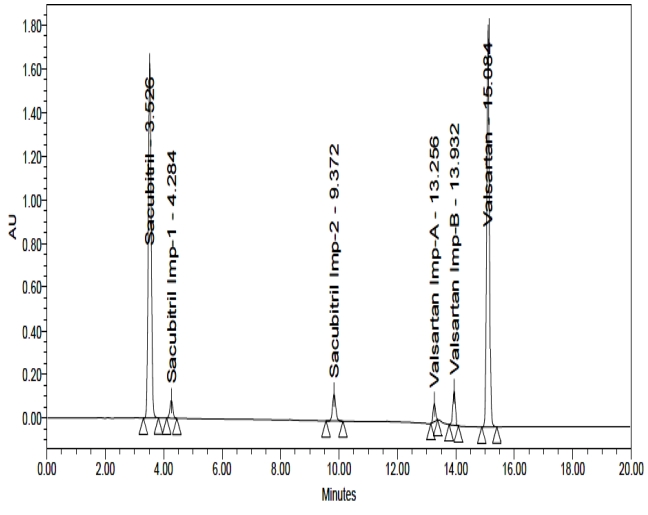

Fig. 8: Typical chromatogram of system suitability

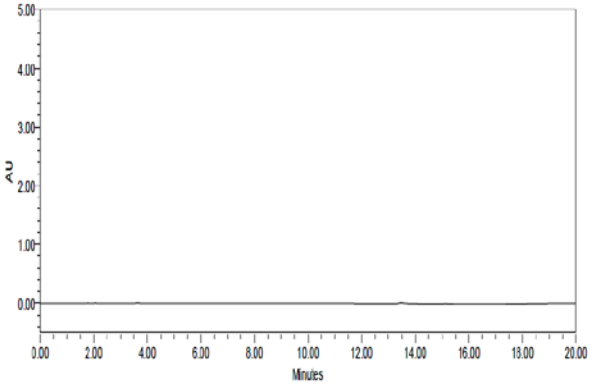

Fig. 9: Chromatogram for blank

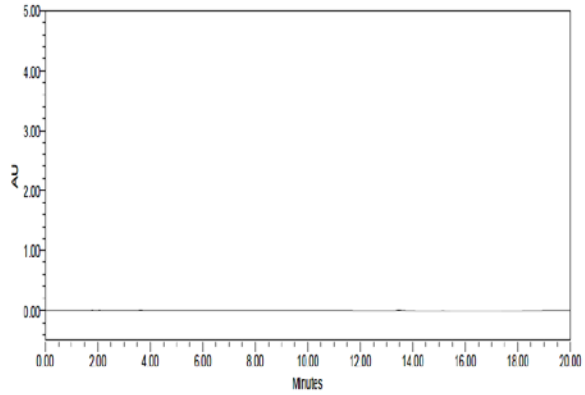

Fig. 10: Chromatogram for Placebo

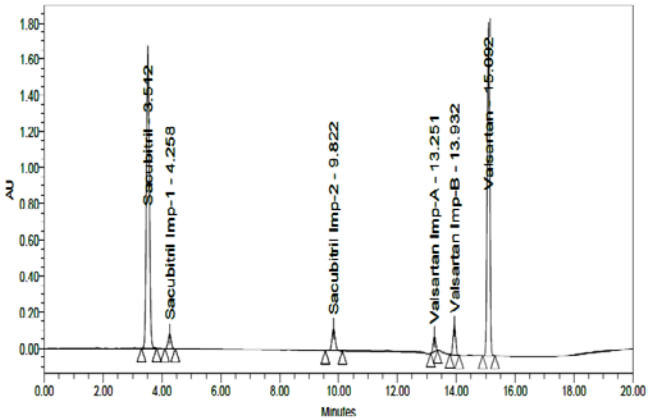

Fig. 11: Chromatogram of standard solution

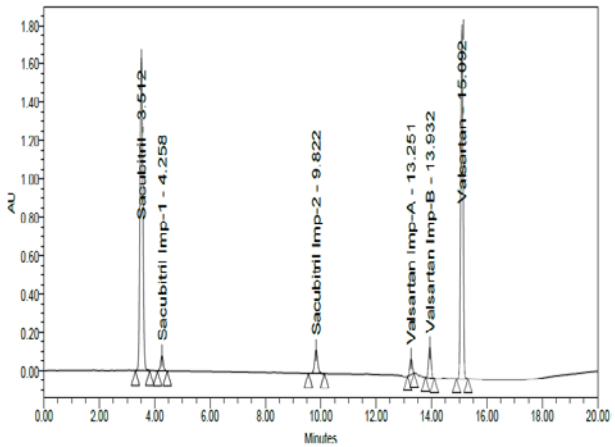

Fig. 12: Chromatogram of sample

Table 9: Accuracy results for Sacubitril

S. No.	% Level	% Recovery	Ave % Recovery
1	50	98.65	98.88
2		99.12	
3		98.87	
4	100	99.89	98.79
5		99.12	
6		97.36	
7	150	99.14	98.71
8		98.63	
9		98.35	

Table 10: Accuracy results for Valsartan

S. No.	% Level	% Recovery	Ave % Recovery
1	50	99.36	99.12
2		98.87	
3		99.12	
4	100	98.14	99.01
5		99.36	
6		99.54	
7	150	99.87	99.21
8		99.21	
9		98.53	

Table 11: Accuracy results of Sacubitril Imp-1

S. No.	% Level	% Recovery	Ave % Recovery
1	50	98.35	98.65
2		98.47	
3		99.12	
4	100	99.35	99.12
5		99.14	
6		98.87	
7	150	99.17	99.15
8		99.54	
9		98.74	

Table 12: Accuracy results of Sacubitril Imp-2

S. No.	% Level	% Recovery	Ave % Recovery
1	50	99.66	99.06
2		98.78	
3		98.75	
4	100	97.65	97.27
5		97.84	
6		96.33	
7	150	98.12	98.29
8		98.42	
9		98.33	

Table 13: Accuracy results of Valsartan Imp-A

S. No.	% Level	% Recovery	Ave % Recovery
1	50	98.53	98.06
2		97.39	
3		98.26	
4	100	99.10	98.54
5		98.89	
6		97.63	
7	150	98.64	98.04
8		98.12	
9		97.36	

Table 14: Accuracy results of Valsartan Imp-B

S. No.	% Level	% Recovery	Ave % Recovery
1	50	98.45	98.32
2		98.17	
3		98.33	
4	100	97.54	97.87
5		98.41	
6		97.66	
7	150	98.36	98.05
8		98.12	
9		97.68	

Table 15: Forced Degradation results for Sacubitril

Degradation Condition	% of Purity	% of Degradation	Purity Angle	Purity Threshold
Unstressed Degradation	99.9	-	0.145	5.028
Acid Degradation	88.56	11.34	0.142	5.032
Alkali Degradation	85.31	14.59	0.138	5.124
Peroxide Degradation	82.98	16.92	0.156	5.236
Reduction Degradation	83.69	16.21	0.168	5.039
Thermal Degradation	86.35	13.55	0.172	5.051
Photolytic Degradation	88.49	11.41	0.189	5.044

Table 16: Forced Degradation results for Valsartan

Degradation Condition	% of Purity	% of Degradation	Purity Angle	Purity Threshold
Unstressed Degradation	100.0	-	0.087	5.008
Acid Degradation	85.33	14.67	0.089	5.005
Alkali Degradation	87.68	12.32	0.094	5.028
Peroxide Degradation	80.49	19.51	0.064	5.124
Reduction Degradation	85.33	14.67	1.102	5.051
Thermal Degradation	88.18	11.82	1.025	5.041
Photolytic Degradation	85.16	14.84	1.001	5.024

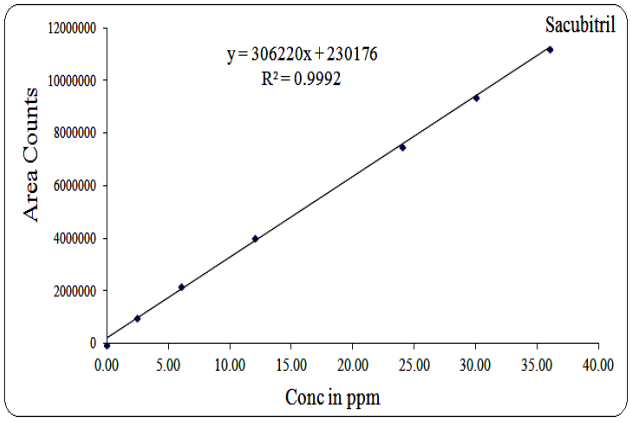

Fig. 13: Linearity plot for Sacubitril

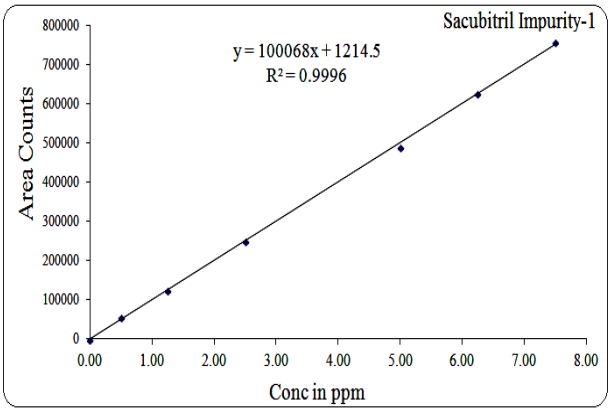

Fig. 14: Linearity plot for Sacubitril Imp-1

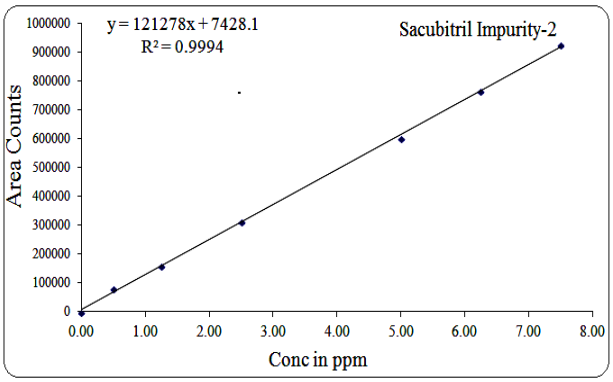

Fig. 15: Linearity plot for Sacubitril Imp-2

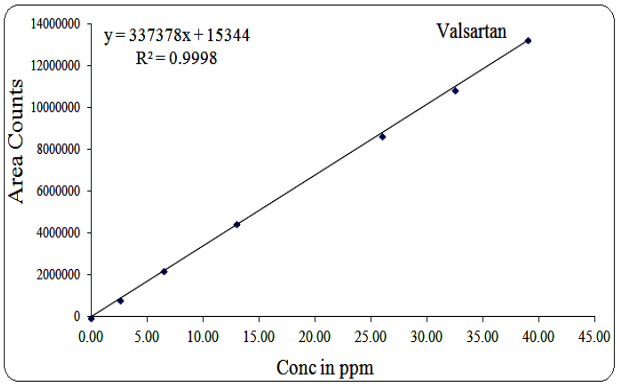

Fig. 16: Linearity plot for Valsartan

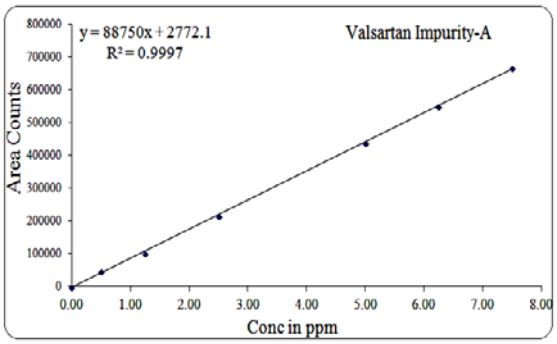

Fig. 17: Linearity plot for Valsartan Imp-A

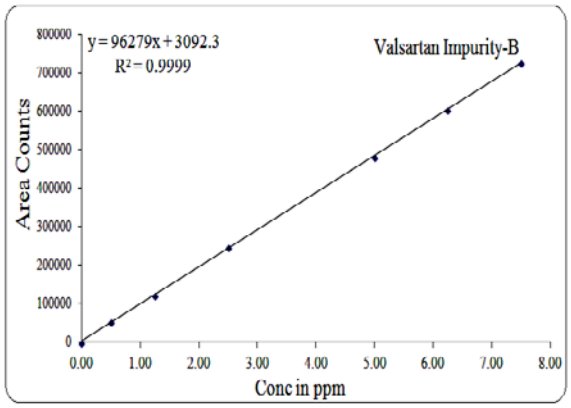

Fig. 18: Linearity plot for Valsartan Imp-B

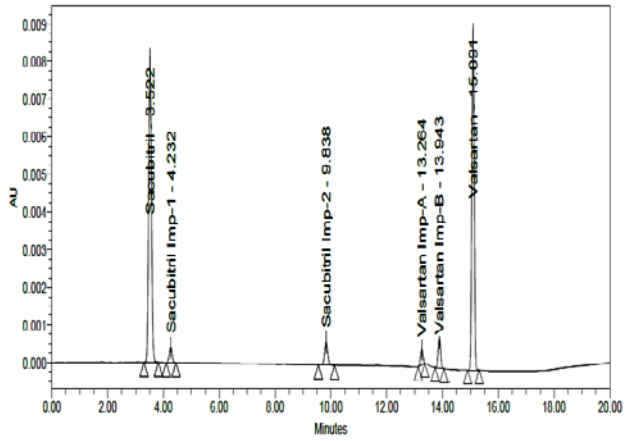

Fig 19: Chromatogram of LOD

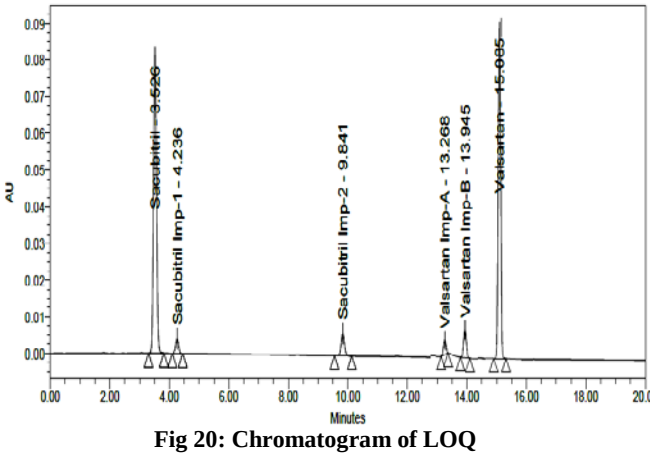

Fig 20: Chromatogram of LOQ

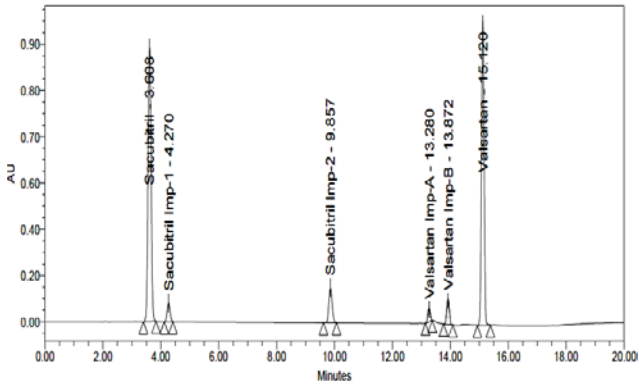

Fig 21: Chromatogram of Accuracy 50%

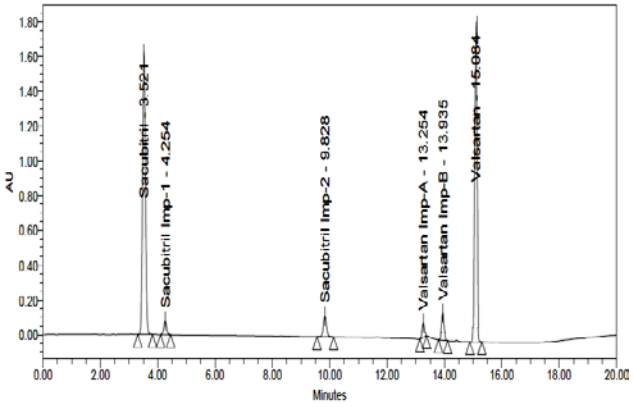

Fig 22: Chromatogram of Accuracy 100%

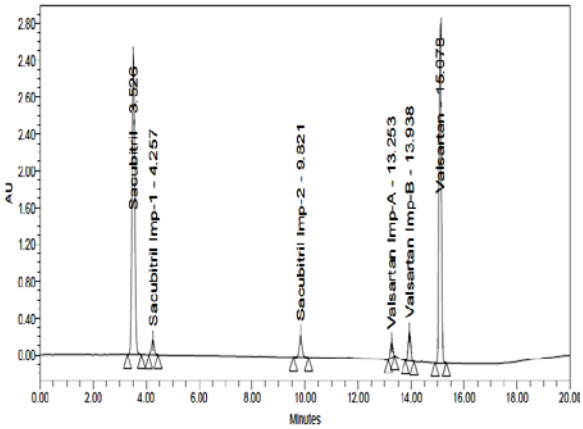

Fig 23: Chromatogram of Accuracy 150%

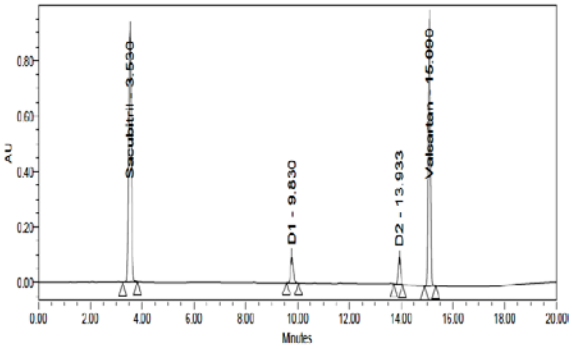

Fig 24: Chromatogram for Acid degradation

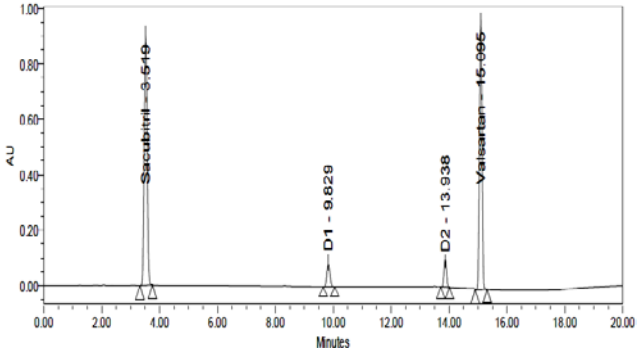

Fig 25: Chromatogram for Alkali degradation

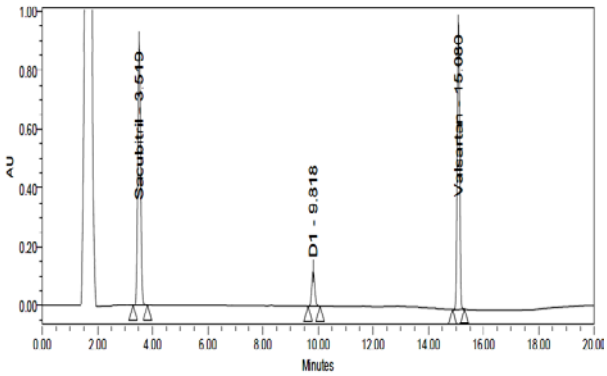

Fig 26: Chromatogram for Peroxide degradation

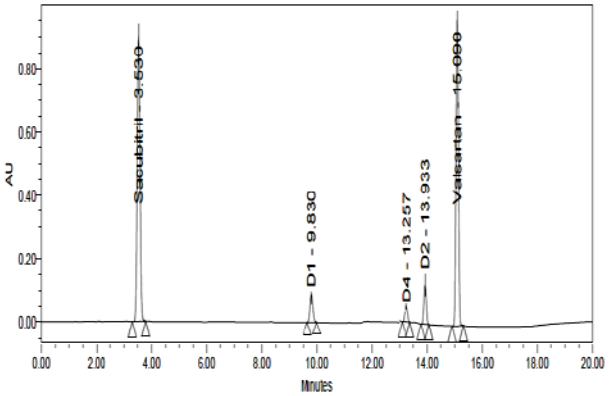

Fig 27: Chromatogram for Reduction degradation

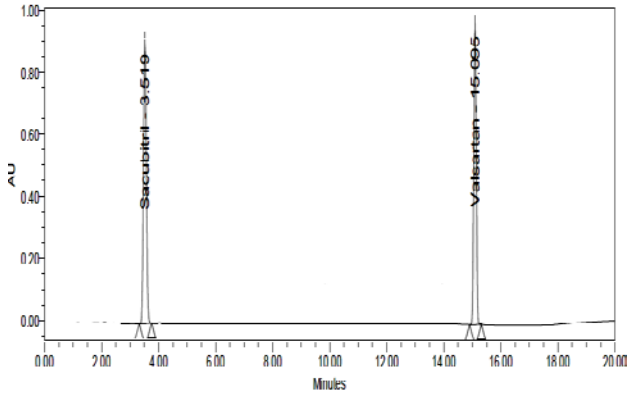

Fig 28: Chromatogram for Thermal degradation

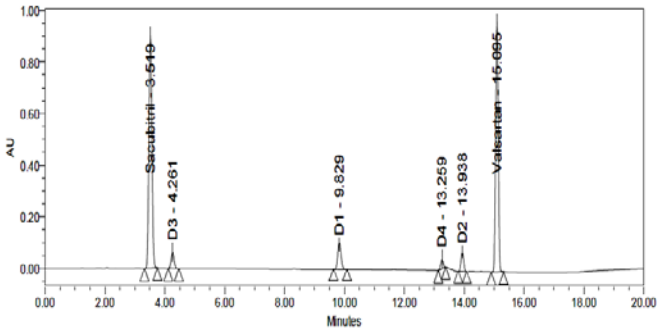

Fig 29: Chromatogram for Photolytic degradation

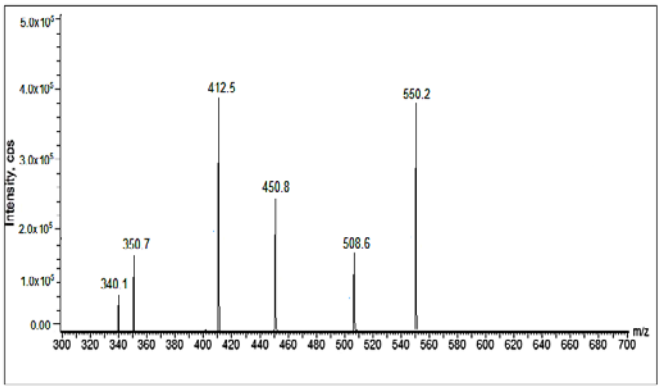

Fig 30: LC-ESI-MS/MS spectrum of $[M+H]^+$ ions of Sacubitril Imp-1(m/z 550)

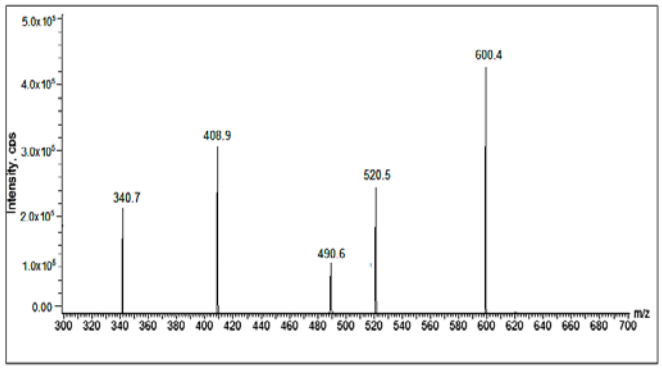

Fig 31: LC-ESI-MS/MS spectrum of $[M+H]^+$ ions of Sacubitril Imp-2(m/z 600)

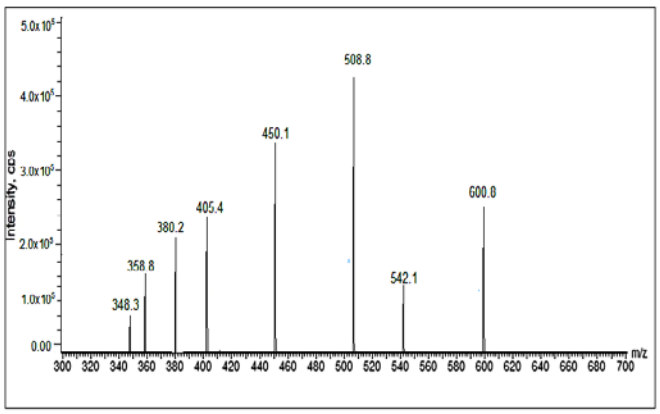

Fig 32: LC-ESI-MS/MS spectrum of $[M+H]^+$ ions of Valsartan Imp-A(m/z 542)

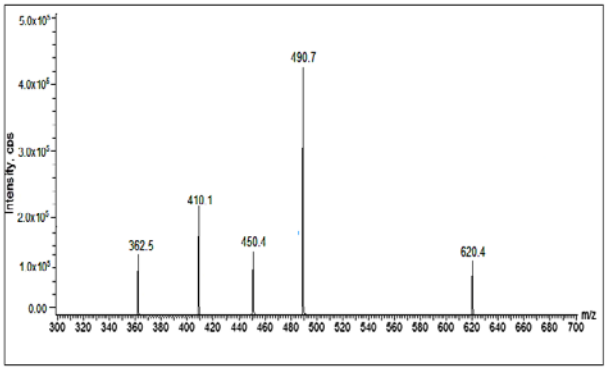

Fig 33: LC-ESI-MS/MS spectrum of $[M+H]^+$ ions of Valsartan Imp-B (m/z 620)

Degradation Effects and its characterization

The Sacubitril and Valsartan sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Forced degradation studies were performed to show the method is suitable for degraded products. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities.

Acid Degradation

In acid degradation Sacubitril and Valsartan having two degradation products D1 and D2. The retention times of D1 and D2 detected by LC-MS was at 9.83 and 13.93min. These degradation products are Sacubitril Impurity-2 and Valsartan Impurity-B conformed due to RT's and their m/z values are 600, 620. Acid degradation procedure is 5ml of sample transferred into a 50ml volumetric flask and add 1ml of 1N HCl heat for 30min at 60°C after that add 1ml of 0.1N NaOH then makeup to mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Alkali Degradation

In alkali degradation Sacubitril and Valsartan having two degradation products D1 and D2. The retention times of D1 and D2 detected by LC-MS was at 9.83 and 13.94min. These degradation products are Sacubitril Impurity-2 and Valsartan Impurity-B conformed due to RT's and their m/z values are 600,620. The degradation procedure was performed as follows 5ml of sample transferred into a 50ml volumetric flask add 1ml of 1N NaOH heat for 30min at 60°C after that add 1ml of 1N HCl then make up to the mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Peroxide Degradation

In peroxide degradation Sacubitril and Valsartan having one degradation product D1 is formed. The retention times of D1 detected by LC-MS was at 9.82 min. These degradation products are Sacubitril Impurity-2 conformed due to RT and their m/z value are 600. The degradation procedure was performed as follow 5ml of sample transferred into a 50ml volumetric flask add 1ml of 30% H_2O_2 heat for 30min at 60°C then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Reduction Degradation

In reduction degradation Sacubitril and Valsartan having three degradation products D1, D2 and D3 are formed. The retention times of D1, D2 and D4 are detected by LC-MS was at 9.81, 13.93and 13.25 min. These degradation products are Sacubitril Impurity-2 and Valsartan Impurity-1, 2 conformed due to RT and their m/z values are 600, 542 and 620. The degradation procedure was performed as follows 5ml of sample transferred into a 50ml volumetric flask add 1ml of 10% sodium bicarbonate solution heat for 15min at 60°C then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Thermal Degradation

In thermal degradation there is no degradation products are formed. The degradation was performed as follows, drug sample solution was placed in oven at 105°C for 6hr. Then resultant solution was injected into HPLC system.

Photolytic Degradation

In photolytic degradation total four degradation products D1, D2 and D3, D4 are formed. The retention times of D1, D2 and D3, D4 are detected by LC-MS was at 9.82, 13.93, 4.26 and 13.25 min. These degradation products are Sacubitril Impurity-1, 2 and Valsartan Impurity-A, B due to their RT's and their m/z values are 600, 620, 550 and 542. In degradation procedure sample solution was exposed into sunlight for 12hr. The sample was injected into HPLC system.

4. CONCLUSION

Finally this method having good resolution of Sacubitril and Valsartan and their impurities with short runtime, high effictive and comply with System suitability specifications of ICH guidelines. This method was conceived to incredible, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Sacubitril and Valsartan. The result of Sacubitril and Valsartan applying different stress condition stable in thermal conditions. It degraded extensively under acidic, alkali and photolytic conditions. The LC-MS/MS results were used to characterization of resultant degradation products. Obtained stability studies, degradation studies are used to more comprehend to Sacubitril and Valsartan during storage and stable formulations and contribute to the safety of Sacubitril and Valsartan being manufactured in pharmaceutical laboratories.

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Conflicts of interest

The authors declare that there is no conflict of interests regarding to publication of paper.

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