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Original Article

DEVELOPMENT OF ASSAY METHOD AND FORCED DEGRADATION STUDY OF VALSARTAN AND SACUBITRIL BY RP-HPLC IN TABLET FORMULATION

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ABSTRACT

Objective: A stability-indicating high performance liquid chromatographic (HPLC) method was developed and validated for the estimation of combined tablet formulation of valsartan and sacubitril.

Methods: Chromatographic separation was optimized by gradient HPLC on a C18 column [Xterra, 250 x 4.6 mm, 5µ] utilizing a mobile phase consisting acetonitrile, methanol and potassium dihydrogen phosphate, pH 3.8 in the ratio of 30: 50:20 v/v at a flow rate of 1 ml/min with UV detection at 263 nm.

Results: The retention time of sacubitril and valsartan was 3.01 min and 4.22 min respectively. Good linearity obtained over the range of 20µg/ml to 160µg/ml for valsartan and sacubitril. The correlation coefficient was found to be 0.999and0.998 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 limits.

Conclusion: 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: Degradation study, HPLC method, Sacubitril, Valsartan

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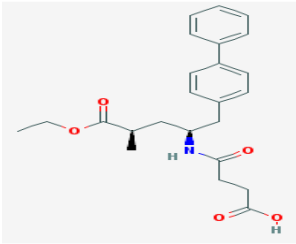
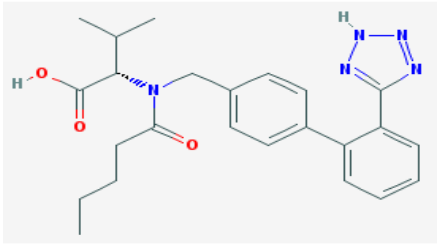
INTRODUCTION

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 [1-5]. Methods such as HPLC [6-8], LC-MS [9-11], Protein precipitation [12], Capillary electrophoresis [13] and Simultaneous UV-spectrophotometric methods [14-15] 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 [16]. Sacubitril is an antihypertensive drug used in combination with valsartan for the treatment of heart failure. [17-19] Literature search reveals that only two analytical methods were reported for simultaneous estimation of valsartan and sacubitril from rat plasma using LC-MS/MS [20] and from a synthetic mixture using HPLC [21]. There is no stability indicating analytical methods were reported for simultaneous estimation of atovaquone and proguanil. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for the simultaneous estimation of valsartan and sacubitril from bulk and pharmaceutical dosage form.



Sacubitril structure from pubchem



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MATERIALS AND METHODS [21]

Chemicals and reagents

HPLC grade methanol, acetonitrile and analytical grade trifluoro 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.

Instrumentation

The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. HPLC column BDS 250 mm 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 sample solution

Twenty tablets each containing 24 mg 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 1 ml of solution into a 10 ml volumetric flask

and diluted up to the mark with diluents. to obtain a Final concentration of sacubitril and valsartan was found to be 24 and 26µg/ml respectively.

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 263 nm. Separation was carried out in isocratic mode on Xterra C18 column (4.6x250 mmx5 µm) and the retention time of sacubitril and valsartan was found to be 3.01 min and 4.22 min respectively. (fig. 1), using mobile phase consisting acetonitrile, methanol and potassium dihydrogen phosphate, pH 3.8 in the ratio of 30: 50:20 v/v at a flow rate of 1 ml/min with UV detection at 263 nm. The mobile phase filtered through nylon millipore (0.2 µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultrasonicator prior to use. Chromatography was carried out at room temperature 25 °C and maintains the column temperature at 32 °C.

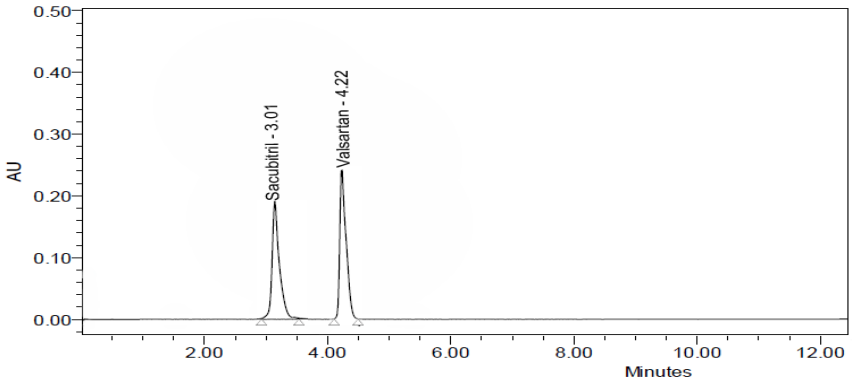

Fig. 1: Chromatogram of sacubitril and valsartan

The developed Method was validated for linearity, precision, accuracy, ruggedness and is applied for forced degradation studies as per the ICH guidelines [22-26**]**.

RESULTS AND DISCUSSION

Method validation

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. The correlation coefficient was found to be 0.999and0.998 for sacubitril and valsartan respectively (shown in fig 2 and 3).

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

The LOD is calculated using the formula 3.3 times σ/s where "σ" is the standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve. Similarly, LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ are shown in table 2 and 3.

Table 1: Linearity data for valsartan and 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

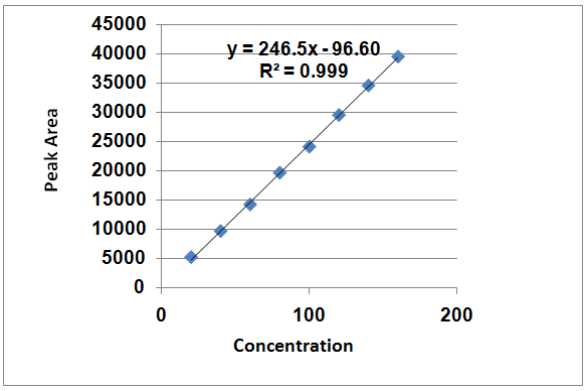

Fig. 2: Calibration curve of sacubitril

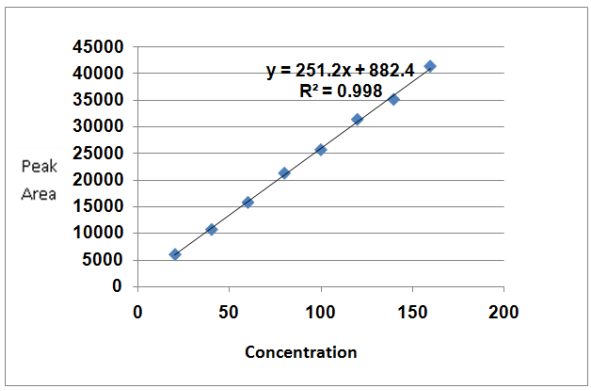

Fig. 3: Calibration curve of valsartan

Table 2: LOD and LOQ results of sacubitril

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg area
20	5373	5198	5281	5284
40	9826	9613	9746	9728
60	14119	14290	14372	14260
80	19492	19124	20452	19689
100	23865	23677	24865	24135
120	29238	29148	30238	29541
140	34611	34521	34611	34581
160	39184	39284	40084	39517
Intercept	-18.57	-228.9	-41.57	-96.6
slope	244.2	245.4	249.9	246.5
Intercept standard deviation				115.36
LOD (µg/ml)				1.54
LOQ(µg/ml)				4.68

Table 3: LOD and LOQ results of valsartan

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg Area
20	6039	5997	6087	6041
40	10178	11012	11013	10734
60	15817	15675	16057	15849
80	21156	21346	21541	21347
100	25195	26101	25987	25761
120	31034	31274	32114	31474
140	35273	35192	35287	35250
160	41312	41178	42018	41502
Intercept	639.2	1040	968.1	882.4
Slope	251.2	249.2	253.2	251.2
Intercept standard deviation				213.69
LOD (µg/ml)				2.80
LOQ(µg/ml)				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 (table 4) and recording the chromatograms of six standard solutions. The % RSD of Sacubitril and Valsartan was found to be 0.31 and 0.27 respectively.

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 0.31 and 0.27 respectively (table 5).

Table 4: Method precision data of valsartan and sacubitril

S. No	Sacubitril (40µg/ml)	Valsartan (140µg/ml)
	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

Table 5: Precision data for sacubitril and 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	0.31	0.27	0.27	0.27	0.27

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 the pure drug at three different concentration levels each in triplicate. Mean percentage recovery values at three different concentrations of the two drugs were calculated. The % mean recovery of sacubitril (99.20-99.54%) and valsartan (99.85-100.90. %) at each level was within the limits of 98% and 102% (table 6).

Table 6: 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	80	79.43	99.29	99.43	0.14	0.15
3	80	79.66	99.58			
1	160	159.38	99.61			
2	160	158.96	99.35	99.54	0.16	0.17
3	160	159.44	99.65			
1	240	240.05	100.02			
2	240	236.88	98.70	99.20	0.71	0.72
3	240	237.35	98.89			
Accuracy of valsartan						
S. No.	**Conc.**	**Calculated concn.**	**% recovery**	**Mean recovery**	**SD**	**%RSD**
1	80	80.45	100.56			
2	80	80.55	100.69	100.90	0.48	0.48
3	80	81.16	101.45			
1	160	160.86	100.53			
2	160	160.98	100.61	100.57	0.038	0.04
3	160	160.92	100.57			
1	240	239.83	99.92			
2	240	239.51	99.79	99.85	0.069	0.07
3	240	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. 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 7), which is within acceptance limits.

Table 7: 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 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 (table 10 and 11)

Acid degradation studies

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

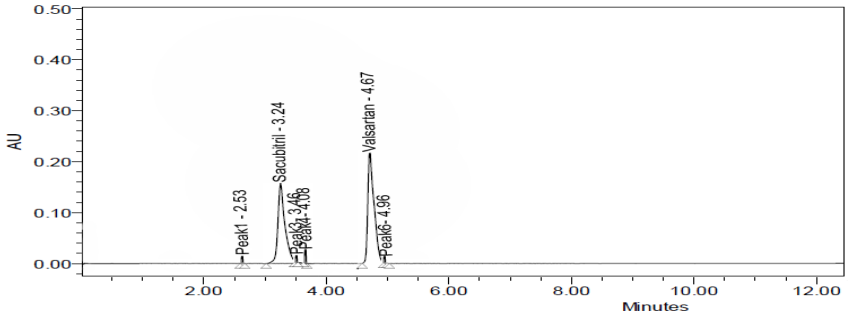

Fig. 4: Chromatogram of acid degradation

Alkali degradation studies

To 1 ml of stock solution of standard drug and sample valsartan and sacubitril, 1 ml of 2N NaOH was added and refluxed for 30 min at 60 °c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of the sample. (fig. 5).

Oxidative degradation

To 1 ml of stock solution of standard drug and sample of valsartan and sacubitril, 1 ml of 20% $H_2O_2$was added and refluxed for 30 min at 60 °c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample (fig. 6).

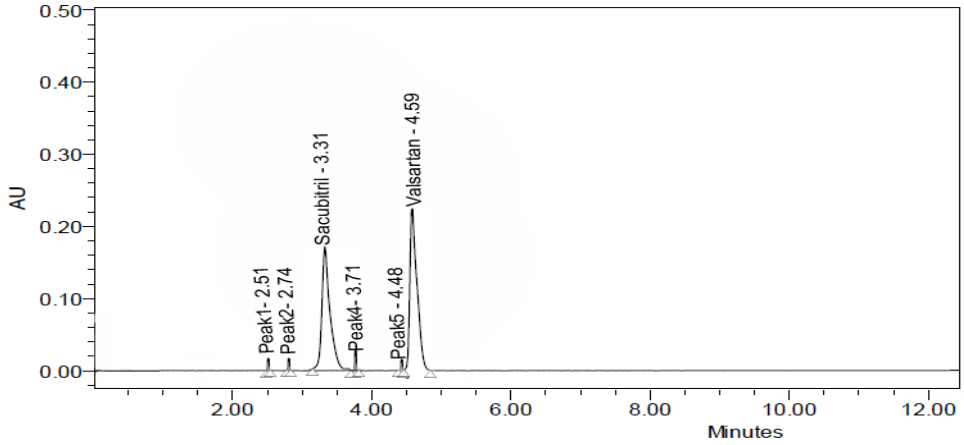

Fig. 5: Chromatogram of base degradation

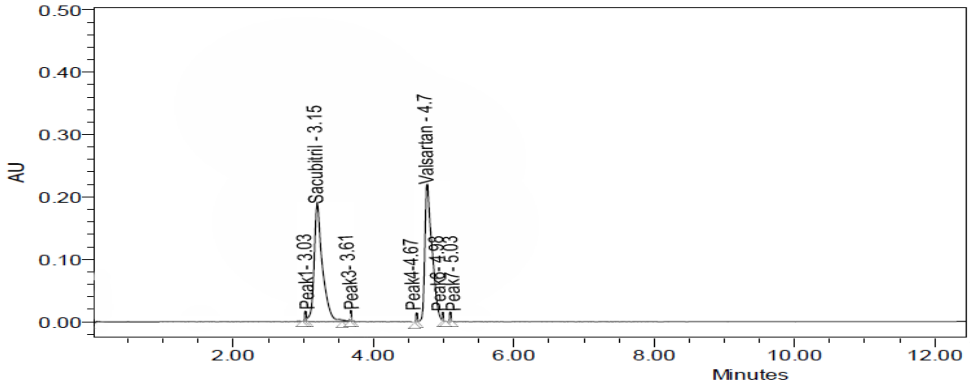

Fig. 6: Chromatogram of oxidative degradation

Photostability studies

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

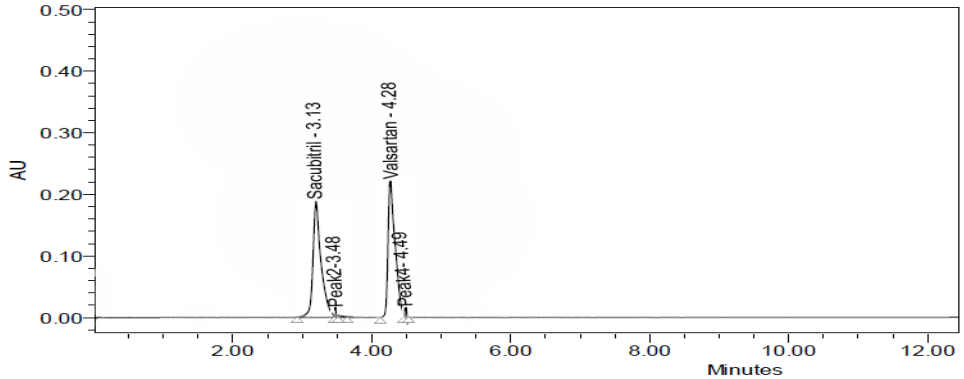

Fig. 7: Chromatogram of UV degradation

Thermal degradation studies

The 1 ml of stock solution of standard drug and sample of valsartan and sacubitril was exposed to temperature 105 °C for 24 h for HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample (fig. 8).

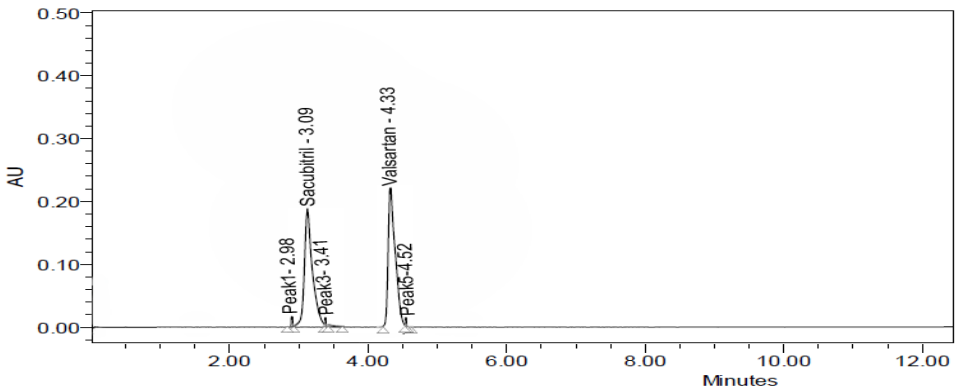

Fig. 8: Chromatogram of thermal degradation study

Table 8: Results of stress degradation studies of sacubitril

S. No.	Stress conditions	Time	% assay	% degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	91.2	8.8	0.17	0.21
2	Base Degradation	30 min	92.6	7.4	0.16	0.22
3	Peroxide Degradation	30 min	97.5	2.5	0.22	0.25
4	UV Degradation	7 d	96.6	3.4	0.21	0.28
5	Thermal Degradation	24 h	97.8	2.2	0.14	0.18

Table 9: Results of stress degradation studies of valsartan

S. No.	Stress conditions	Time	% assay	% degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	97.3	2.7	0.12	0.16
2	Base Degradation	30 min	97.1	2.9	0.19	0.25
3	Peroxide Degradation	30 min	91.7	8.3	0.16	0.20
4	UV Degradation	7 d	95.8	4.2	0.17	0.23
5	Thermal degradation	24 h	98.4	1.6	0.19	0.26

Valsartan and sacubitril 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, a 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 a sample of the pharmaceutical dosage form.

CONCLUSION

In present study valsartan and sacubitril simultaneously estimated by HPLC, good linearity obtained for both drugs (20µg/ml-160µg/ml) with Correlation coefficient of 0.999and0.998 for sacubitril and valsartan respectively. The results for precision, recovery and ruggedness were within limits. Hence the method was successfully applied for degradation studies, the developed stability indicating HPLC-UV method for simultaneous estimation of valsartan and sacubitril was novel, simple, precise, accurate, robust and cost-effective method. There is no HPLC method reported till now on selected combination drugs. Hence the developed method suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

CONFLICT OF INTERESTS

Declare none

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