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Stability indicating analytical method development and validation for estimation of Sacubitril and Valsartan in bulk and pharmaceutical dosage form using RP-HPLC

Farheen Begum[1]*, S.H. Rizwan[2]

Deccan School of Pharmacy, Aghapura, Dar-Us-Salam, Hyderabad, Telangana

Corresponding Author: Farheen Begum

Email: farheen.safoora01@gmail.com

ABSTRACT

A new simple, rapid, specific, accurate and precise stability indicating RP-HPLC method has been developed for the estimation of Sacubitril and Valsartan in bulk & pharmaceutical dosage form. From UV spectrophotometric method selected wavelength for estimation of drugs were 241 nm as isobestic point and 230 nm, 228 nm as λ max of SAC and VAL respectively by using methanol as a solvent. RP-HPLC method was developed by using Inertsil ODS (250×4.6mm) 5µ. The samples were analyzed by using mixed phosphate buffer (P^H adjusted to 3 using orthophosphoric acid): Methanol: Acetonitrile (30:50:20 % v/v) as the mobile phase at the flow rate of 1.0 ml/min and detection wavelength is 241 nm. Both the drugs were eluted within 5 minutes and gave sharp peaks with high theoretical plate count and low tailing factor. The retention time for Sacubitril and Valsartan was found to be 2.927min and 4.003 min respectively. Calibration curve was linear with correlation coefficient of 0.997 and 0.997 over a concentration range of 58.8-137.2 µg/ml and 61.2-142 µg/ml for Sacubitril and Valsartan respectively. The percent recovery was 99.60and 98.08for Sacubitril and Valsartan respectively indicating accuracy and reliability of the method. Forced degradation studies were carried out and drug peaks were well resolved without any significant degradation products when subjected to stress conditions. So the developed stability indicating method could be utilized for routine analysis of Sacubitril andValsartan in bulk and pharmaceutical dosage form.

Keywords: Sacubitril,Valsartan , Stability Indicating, UV, RP-HPLC.

INTRODUCTION

Valsartan is chemically named as (2S)-3[N-({4[-2(2H-1,2,3,4 tetrazol-5-yl)phenyl]phenyl}-methyl) pentanamido] butanoic acid. Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease [4-5].

Sacubitrilis chemically named as3-{[(2S,4R)-1-{[1,1'-biphenyl]-4-yl}-5-ethoxy-4-methyl-5-

oxopentan-2-yl]carbamoyl}propanoicacid. [1-3] Sacubitril is a prodrug that is activated to sacubitrilat (LBQ657) by de-ethylation via esterases. Sacubitrilat inhibits the enzyme neprilysin, which is responsible for the degradation of atrial andbrain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume.Sacubitril is a neprilysin inhibitor and is used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV).The combination drug, sacubitril/valsartan (Entresto) is used in place of an ACE inhibitor or ARB. It was approved under the FDA's priority review process for use in heart failure on July 7, 2015. [5-6] The review of literature revealed that several analytical methods have been reported for valsartan in spectrophotometry, HPLC, LC/MS individually and in the combination with other drugs [6-12]. To date there have been no published reports about the stability indicating method for estimation of sacubitril and valsartan by HPLC in bulk and in pharmaceutical dosage forms. This present study report for the first time stability indicating simultaneous estimation of sacubitril and valsartan by RP-HPLC in bulk drug and in pharmaceutical dosage form.

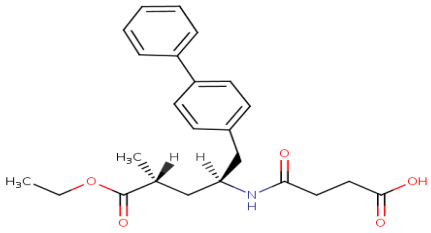

Fig 1: Structure of Sacubitril

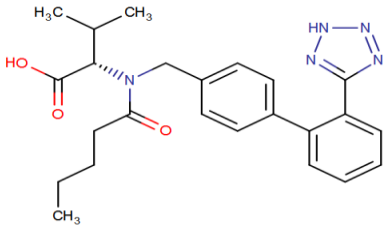

Fig 2: Structure of Valsartan

EXPERIMENTAL WORK

Chemicals

Sacubitril and valsartan were obtained as gift sample from Chandra Pharma Research Laboratory in Hyderabad and marketed formulation was purchased from local market.

Instrument and chromatographic condition

All solvent used in this work are HPLC& AR grade. Instrument and chromatographic condition RP-HPLC Agilent 1220 infinity seriesseparation model equipped with UV Detector was employed in this method. The EZchrom software was used for LC peak integration alongdata acquisition and data processing. The column used for separation of analyte isInertsil ODS (250×4.6mm) 5µ. Mobile phase consisting of phosphate buffer: Methanol: Acetonitrile in the ratio of30:50:20 % v/v at flow rate 1 ml/min. it was filter through 0.45 µm nylon filter and sonicated for 5 min in ultrasonic bath. Sample was analyzed at 241 nm at an injection volume of 20µl.

Preparation of phosphate buffer

1.625 gm of Potassium Di Hydrogen ortho phosphate and 0.3 gms of Di Potassium Hydrogen ortho phosphate was weighed and dissolve in 100 ml of water and volume was made up to 550 ml with water. Adjust the P^H using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Solutions

Preparation of Mixed Standard Solution: (98 µg/ml &102 µg/ml)

Weigh accurately 98mg of Sacubitril&102 mg of Valsartan and dissolve in 100 ml of diluents. From the above stock solution 98 µg/ml of Sacubitril and 102 µg/ml ofValsartanis prepared by diluting 1 ml to 10 ml with mobile phase. This solution (98 µg/ml of Sacubitril & 102 µg/ml of Valsartan) is used as working standard concentration for recording chromatogram.

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 500 ml of volumetric flask. Add sufficient quantity of diluents & sonicated for 5 minutes further the volume is made up with diluents and filtered. From the filtered solution 1 ml was pipette out into a 10 ml with diluents.

Table 1: Summary of Chromatographic conditions

S. No	Parameter	Description
1.	Column	ODS Inertsil C-18 (250×4.6mm) 5µ
2	Mobile Phase & P^H	Phosphate Buffer: Methanol: Acetonitrile p^H 4, 30:50:20 v/v
3	Flow rate	1 ml/min
4	Column & sample temperature	Room temperature (20-25^{o}C)
5	Detection Wavelength	241 nm
6	Detector	Photo diode array
7	Injection Volume	20 µl
8	Retention time	Valsartan - 4.003Min ; Sacubitril - 2.927Min
9.	Run time	6 mins.

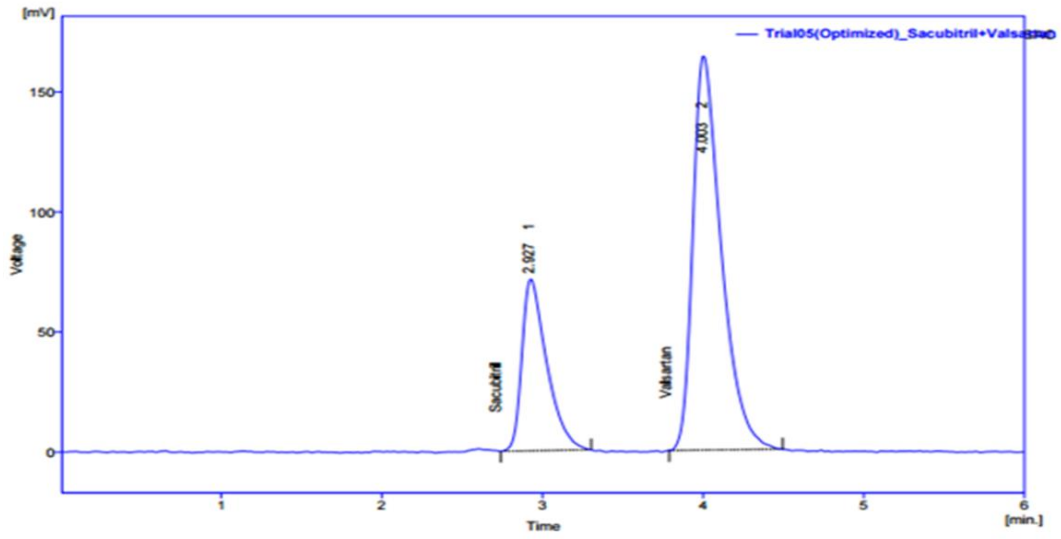

Fig 3: Typical chromatogram of Valsartan and Sacubitril

Method validation

The validation of method was carried out as per ICH guideline. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ. Specificity:Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation product and related substances.

Accuracy

The accuracy was determined by calculating % recoveries of valsartan andsacubitril. It was carried out by adding known amount of each analyte corresponding to three conc. Levels (100, 120, 140) of the label claims to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by proposed method.

Precision

Method precision

Precision of an analytical method is usually expressed as the standard deviation. Method precision was demonstrated by preparing six samples as per test method representing single batch and were chromatographed. The precision of the method was evaluated by computing the %RSD of the results. The individual values and the low % RSD observed on the values are within acceptance criteria and indicates that method is precise.

Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits linear responses and directly proportional over the relevant conc. Range for the target conc. of the analyte. The linear regression data for the calibration plot is the indicative of a good linear relationship between peak and concentration over wide range. The correlation coefficient was indicative of high significance.

Robustness

Robustness of method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates &finally the method was found to be robust.

Ruggedness

The ruggedness of the method was studied by determining the analyst to analyst variation by performing the Assay by two different analysts. % RSD Assay values between two analysts not greater than 2.0%, hence the method was rugged.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable responses and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve.

Assay of valsartan and sacubitril in pharmaceutical dosage form

Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP –HPLC system. The sample solution was scanned at 241 nm. The percentage drug estimated was found to be 100.17% and 100.55% respectively as sacubitril and valsartan. The chromatogram showed two single peaks of sacubitril and valsartan was observed with retention time 2.927min and 4.003 min respectively.

Forced degradation studies

Stress studies are performed according to ICH guidelines under following conditions.

Acid degradation

To 5 ml of sample solution 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.

Alkaline degradation

To 5 ml of sample solution add 1 ml of 0.1 N NaOH 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.

Peroxide degradation

To 5 ml of sample solution 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.

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.

Thermal degradation

Expose about 100 mg of sample in to dry heat at 80^0C 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.

Record the peak area of stressed samples then compare it with peak area of unstressed sample to determine the % degradation.

$$\% \text{ degradation} = \frac{\text{Response of unstressed sample} - \text{response of stressed sample}}{\text{Response of unstressed sample}} \times 100$$

RESULT &DISCUSSION

Sacubitril/valsartan (Entresto) is a recent combination in the market usedto reduces the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV). Literature survey reveals that various methods for the estimation of Valsartan individually and in combination with other drug is reported, but no method has been reported for the estimation of the Sacubitril and Valsartan in combine dosage form by RP-HPLC method. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for estimation of Sacubitril and Valsartan from combine dosage form using HPLC. Specific objectives includes

The conditions in HPLC were optimized in order to obtain the drugs separation of eluted compounds. Initially various composition of mobile phase & PH range were tried in order to have a good separation of the titled ingredients. The composition of Mobile phase & PH selections were based on peak parameters like height, tailing, capacity factor, symmetric factor, theoretical plates, run plates, run time & resolution. The system with phosphate buffer: methanol: acetonitrile of 30:50:20 was found to be robust with PH 3. The optimum wavelength for detection was 241 nm at which both drugs have good response. Sacubitril was eluted at 2.927 min and Valsartan was eluted at 4.003min respectively.

System suitability test were carried out on a freshly prepared stock solution % RSD of peak area of six replicated injections of standards were taken & was found to be less than 2%. Specificity of the method developed was performed & it was found that there is no interference of excipients with the analyte which indicate that the method is specific for the analysis of the analytes in their dosage form. Assay was determined for both Standard & sample solution & the % assay was found to be 100.17% & 100.55% and respectively.

Calibration curves was found to be linear in the concentration range of 58.8-137.2 µg/ml and 61.2-142 µg/ml for SAC &VAL. Accuracy studies of the method developed wad determined by spiking the known amount of analyte to sample solution and the percentage mean recovery was found to be 99.60% and 98.08% for SAC &VAL respectively. Precision of the method & system were found to be within limits. Limit of detection was found to be 0.72 and 1.56 µg/ml for SAC&VAL respectively and Limit of Quantification was found is to be 2.20 and 4.74µg/ml for SAC &VAL respectively. Robustness of the method was determined by varying flow rate and wavelength & Ruggedness of the method was determined by carrying out the determination by two different analyst.

Samples containing Sacubitril and Valsartan were subjected to various stress conditions and it was found that overall net degradation was within the limits without any significant degradation products.

A RP-HPLC method developed for Sacubitril and Valsartan shows that the results obtained for RP-HPLC are promising with better resolution in set chromatographic conditions. The developed methods were statistically validated which suggested that the methods were within the acceptable limits and hence these methods can be used for the routine determination of Sacubitril and Valsartan in bulk drug and pharmaceutical formulation.

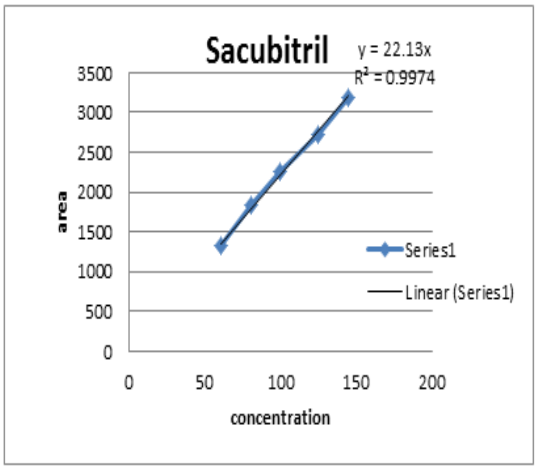

Fig 4: Linearity curve of Sacubitril

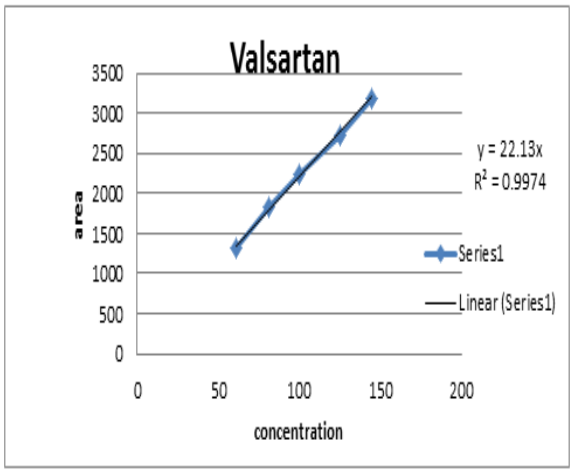

Fig 5: Linearity curve of Valsartan

Table: 2 Results of Linearity

Sacubitril			**Valsartan**	
S.No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	58.8	485.279	61	1331.154
2	78.4	697.105	81	1859.595
3	98	768.173	102	2010.885
4	117.6	1062.233	122	2728.440
5	137.2	1245.814	142	3196.923

Table: 3 Precision method of proposed RP- HPLC method

Sacubitril			**Valsartan**	
S.No.	**Rt**	**Area**	**Rt**	**Area**
1	2.917	765.269	3.993	2012.58
2	2.92	760.464	3.993	2009.765
3	2.923	768.53	3.99	2023.634
4	2.923	763.825	3.987	2028.946
5	2.917	762.172	3.99	2031.51
6	2.93	775.496	4.01	2035.765
Avg	2.921667	765.9593	3.993833	2023.7
Stdev	0.004885	5.42459	0.008232	10.50642
%RSD	0.001672	0.007082	0.002061	0.005192

Table: 4 Recovery data for Sacubitril

Recovery level	**Amount taken (mcg/ml)**	**Accuracy of Sacubitril**				
		Area	Average area	Amount recoverd	%Recovery	Average % Recovery
100%	98	747.051 760.464 768.530	758.681	31.78	99.45	
120%	117.6	1062.233	1057.388	45.95	91.48	

		1050.067				
		1059.864				99.60
140%	137.2	1245.814	1247.078	152.29	107.89	
		1242.233				
		1247.189				

Table: 5 Recovery data for Valsartan

Recovery level	**Accuracy Valsartan**					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	
100%	102	2012.830	2015.409	31.78	100.201	
	102	2009.765				
	102	2023.634				
120%	122.4	2728.440	2738.442	44.76	89.85	
	122.4	2737.172				98.08
	122.4	2749.714				
140%	142.8	3196.923	3175.268	148.78	104.19	
	142.8	3166.488				
	142.8	3162.393				

Table: 6 Robustness data

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				
232nm	2.927	1.267	4.020	1.921
237nm	2.940	1.333	4.050	1.000
241nm	2.940	1.300	4.050	1.000

Table: 7 Result of Ruggedness

Sacubitril	**% Assay**	**Valsartan**	**% Assay**
Analyst 01	99.63	Analyst 01	99.89
Analyst 02	98.33	Analyst 02	98.65
% RSD	1.30%	% RSD	1.24%

Table: 8 Result of LOD and LOQ

DRUG	LOD(µg/ml)	LOQ(µg/ml)
Sacubitril	0.72	2.20
Valsartan	1.56	4.74

Table: 9 Results of Forced Degradation studies.

STRESS CONDITION	PEAK AREA		% DEGRADATION	
	SAC	VAL	SAC	VAL
Unstressed sample	772.321	2014.800	0	0
Acid	775.496	2035.765	0.41	1.04
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

Sacubitril and Valsartan are essential to reduce the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV) so it is therefore necessary to know the quality of these drugs which is possible through the use of simple, sensitive and cost effective analytical methods so that the compounds can be rapidly, routinely and consistently assessed. Literature survey reveals that there are methods to estimate theValsartan individually and with other combinations, but not a single method is reported for estimation of Sacubitril and Valsartan in bulk and multicomponent formulation. So, an attempt was made to develop RP-HPLC method.

A RP-HPLC method proposed as a suitable method for the simultaneous estimation of Sacubitril andValsartan. The chromatographic conditions included mixed phosphate buffer (P^H 3): Methanol: Acetonitrile in the ratio of 30:50:20 % v/v as mobile phase. The optimum wavelength for detection was 241 nm where as SAC was eluted at 2.927 min and VAL was eluted at 4.003 min.

The calibration curves of Sacubitril and Valsartan is linear in the range of 58.8-137.2 µg/ml and 61.2-142 µg/ml respectively. Accuracy study showed the percentage mean recovery of Sacubitril and Valsartan is 99.60% and 98.08% respectively. The amount of Sacubitril and Valsartan present in the taken formulation was found to be 100.17% and 100.55% respectively. Forced degradation studies were carried under various stress conditions. It was found to be stable and the net degradation was within the limits without any significant degradation products. From the above experimental results and parameters it was concluded that, this newly developed methods for the estimation of Sacubitril and Valsartan was found to be simple, rapid, economic, precise, accurate and reproducible. Forced degradation studies carried out are helpful to determine stability of this drug in combination. The analytical technique showed reliable method hence it can be effectively applied for routine analysis in research institutions & quality control department in industries.

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