Section A -Research paper

Sacubitril and Valsartan Analytical Method Development and Validation by RP HPLC in Combined Dosage Form 97/103 mg

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ABSTRACT

Aim &Objective: The objective of this study was to describe a simple and selective LC method for the simultaneous dosing of sacubitril and valsartan in tablet form.

Materials and methods: The chromatographic separations were optimized by isocratic HPLC on a Thermo Scientific Hypurity C18 (100 mm X 4.6 mm), 5 µm, using a mobile phase consisting of 0.1% orthophosphoric acid and a preparation of 500ml of methanol: tetrahydrofuran 500ml. The mobile phase ratio of solution A and solution B was 65:35 (% v/v), flow rate was 1.4 ml/min and UV detection was performed at 254 nm.

Results: The optimized method produced Valsartan and Sacubitril retention times of 4.1 and 5.7 minutes, respectively, with theoretical plate counts and asymmetry within ICH limits. The proposed method has been validated in terms of precision, accuracy, linearity, system suitability, filter validation and solution stability. With Sacubitril, linearity was observed over a concentration range of 10.0-40.0 g/ml (r2 = 0.9992), Valsartan, 10.5-40.0 g/ml (r2 =0.9995) Repeatability analysis showed %RSD less than 2, indicating the method is accurate. Percentile determinations were found to be 99.1% and 99.8%, respectively. This method can be used successfully to evaluate both sacubitril and valsartan, as all statistical evidence supports its efficacy according to ICH criteria.

Conclusions: The proposed method is precise, fast, accurate and can be used for routine quality control analysis.

Keywords: RP-HPLC technique, Sacubitril, valsartan, UV detector, ICH guideline.

INTRODUCTION

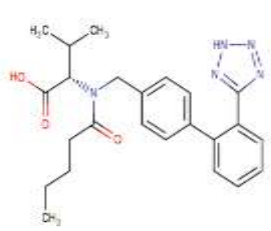
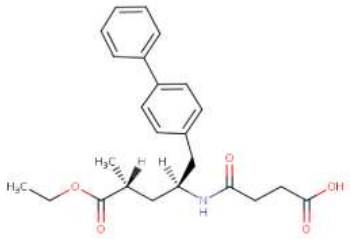
Angiotensin II receptor blocker Valsartan acts on the AT1 receptor and is nonpeptide, orally active, and selective. Valsartan is chemically N-(1- \soxopentyl) -N-[[2'-(1Htetrazol-5- yl) (1Htetrazol-5- yl) [1,1'-biphenyl] -4-yl]methyl]-Lvaline.[1-3] Techniques like protein precipitation, HPLC, LC-MS, and concurrent UV-spectrophotometric procedures are reported for valsartan estimate is used alone or in conjunction with other drugs. Chemically, sacubitril is a 4-[[(2S,4R)-5- ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl)pentan-2-yl]amino]

compound. Acid -4-oxobutanoic. Sacubitril is an antihypertensive drug used in combination with valsartan for the treatment of heart failure.[4-6] Just two analytical techniques have been documented for the simultaneous quantification of sacubitril and valsartan, one from rat plasma using LC-MS/MS and the other from a synthetic combination using HPLC.

There is no analytical methods was developed and validated in the combination of 97/103 mg and also mobile phase is a novel 0.1% orthophosphoric acid solution A and solution B contains in the ratio of methanol and Tetrahydrofuran which shows cost effective and consumption will be less.

By using high dose in heart failure the rapid of action in drug will more and which helps to reduce the morbidity and mortality. So current novel method will useful in future reduces the mortality. And it can used for regular quality control analysis. In table 1 complete description of Sacubitril and Valsartan were tabulated.

Table 1 Description of Valsartan and Sacubitril[7-9]

Description	Valsartan	Sacubitril
Molecular formula	$C_{24}H_{29}N_5O_3$	$C_{24}H_{29}NO_5$
Molecular weight	435.519g/mol	411.49 g/mol
Solubility	Solubility It is almost completely insoluble in water but extremely soluble in methanol and ethanol (99.5% each).	It dissolves readily in water.
Chemical structure		

According to the literature review[10-20], there are few assay methods available, but to the best of our knowledge, a single method for simultaneous assay and determination of Sacubitril and Valsartan in bulk and pharmaceutical dosage forms the combination of dose 97/103 mg is not available. As a result, the current work was undertaken.The proposed method is cost-effective and can be used for routine as well as stability batch analysis in quality control and research laboratories.

MATERIALS AND METHODS

Chemicals & Reagents

Pure samples of Sacubitril and Valsartan Working standard procured from (Man kind Pharma limited).Acetonitrile and THF used a HPLC grade or Equivalent were Purchased from Central drug house Private LTD . Methanol used was of HPLC grade and were purchased from Advent Chembio PVT.LTD. 0.45µm Nylon filters [Thermo Fisher QNN9797919] are used. The tablet samples available in local pharmacy

Instruments Required and chromatographic conditions

Equipment and instruments used in this validation has been calibrated and maintained and will be used within their calibrated period. The weighing operations for this study were performed on the Mettler XS 205 Analytical balance. The Samples were Ultrasonically processed using an Ultrasonicator from

CHEM54/Chemlabs. Chromatographic separation was performed using HPLC with UV detector Aligent Technologies(Waters 2487) by using the Column Agilent Thermo Scientific Hypurity C18 (100mm X 4.6 mm), 5 µm Chromatograms were recorded using the Empower version 3 software (Agilent Technologies) installed on a personal computer. The mobile phase was pumped at the rate of 1.4 mL/minute and the absorption of analytes was detected at 254 nm. Elution mode isocratic. Run time 12 minutes. The experiments were carried out in an air-conditioned laboratory maintained at 25°C±2°C. The amount of standard and sample solutions injected into the HPLC instrument for the analysis was set at 20 µL

Materials required

Volumetric flasks, glass beakers, measuring cylinders, pipettes, 0.45µm Nylon syringe Filters.

Preparation of Mobile phase

Preparation of 0.1% v/v Orthophosphoric acid: Solution A:

Dilute 1mL of Ortho phosphoric acid into 1000mL of MilliQ Water and mix well. Filter this solution through 0.45µm Nylon membrane filter and degas.

Preparation of Solution B:

Prepare a mixture of 500mL of Methanol and 500mL of Tetrahydrofuran in the ratio of 50:50 (%v/v) mix well and degas for 10 minutes.

Preparation of Mobile Phase:

Prepare a mixture of Solution A and Solution B of 65:35 (% v/v) mix well and degas for 10 minutes.

Diluent preparation:

Prepare a mixture of Acetonitrile: Methanol: Water in the ratio (40:40:20), sonicate for 5 minutes and mix well.

Blank Preparation: Use Diluent as a blank**.**

Preparation of Standard Solution

Weigh accurately about 22.0 mg of Sacubitril (equivalent to 9.7 mg Sacubitril) and 22.0 mg of Valsartan (equivalent to 10.3 mg Valsartan) working/reference standard transfer it into a 25 mL volumetric flask, add 10mL of Methanol sonicate for 5minutes to dissolve the contents and dilute to the volume with Methanol and shake well. Pipette out 5 mL of this solution into a 20mL volumetric Flask and Dilute to volume with diluent and shake well. (97µg/ml of sacubitril and 103 µg/ml of valsartan).

Preparation of Sample Solution

Weigh 10 tablets and calculate the average weight. Crush the tablets into fine powder by using mortar and pestle and. Accurately weigh and transfer the tablet powder equivalent to 97mg of Sacubitril and 103 mg of Valsartan (about 416 mg of crushed powder) into a 200 mL volumetric flask, add about 100 mL of diluent and mechanically shaken at 200 RPM for 10 minutes, sonicate for 30 minutes with intermediate shaking, allow it to attain room temperature, then dilute to volume up to the mark with diluent and shake well. Filter the above sample solution through 0.45 µm PVDF syringe filter. Pipette out 5 mL of this clear solution into a 25 mL volumetric flask and dilute to volume with diluent. (97µg/ml of sacubitril and 103µg/ml of valsartan) And obtained results were tabulated in table 2

Table 2 Assay results of valsartan and sacubitril in formulations

Drug Name	Quantity Label Claim (mg)	% Assay
Valsartan	103	99.8
Sacubitril	97	99.1

RESULTS AND DISCUSSIONS

Primarily numerous trials for optimization of method was performed using different phase composition, different organic solvent, different ratio of organic to buffer, different stationary phase and different internal standard chromatographic settings to achieve the finest peak resolution of Sacubitril and Valsartan.

System suitability parameters

System compatibility testing were a crucial component of the LC technique, according to the US Pharmacopoeia, when it came to enhancing the parameters of the suggested approach. For the purposes of demonstrating the applicability of the system, the test solutions for system suitability were injected, and the chromatographic parameters for Sacubitril and Valsartan were assessed.(Table 3)

Table 3 System Suitability study

Name of the parameter	Sacubitril	Valsartan
Retention time (RT)	4.1	5.6
Area (%RSD NMT2.0)	1.7	1.3
Tailing factor (NMT 1.8)	1.4	1.3
Theoretical plates (NLT 2000)	8816	9302

Analytical method validation

The analytical method was optimized and validated in accordance with the current ICH guidelines and to accomplish the vision of specificity, Accuracy, Linearity, Precision, Robustness, Filter validation, solution stability.

1. Specificity

The capacity for unambiguous evaluation of the analyte in the presence of potential contributing factors is known as specificity.

For specificity evaluation: Blank solution, Placebo solution, Sample solution and standard solution were injected into the HPLC system In below the chromatograms obtained in fig 1,2,3,4

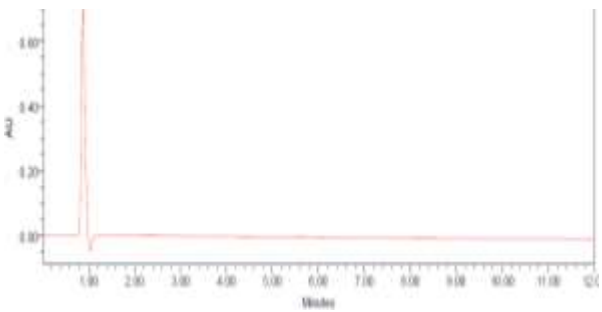


Fig :1 Blank

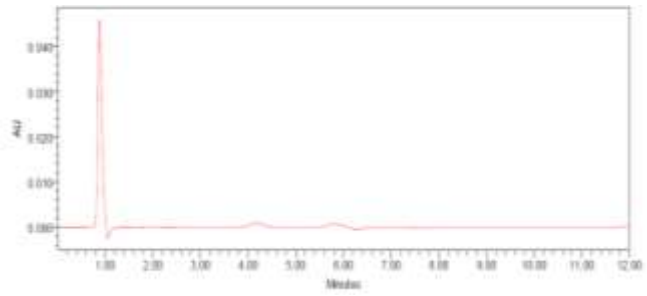


Fig :2 Placebo

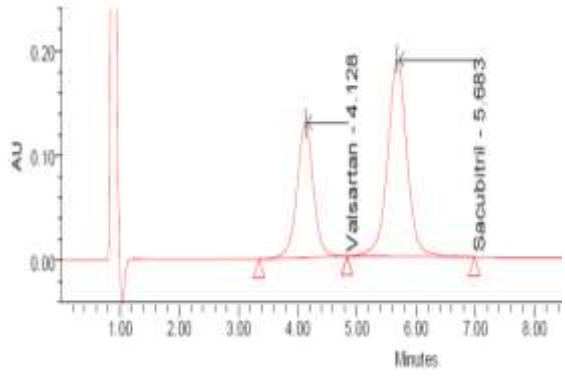


Fig: 3 Standard

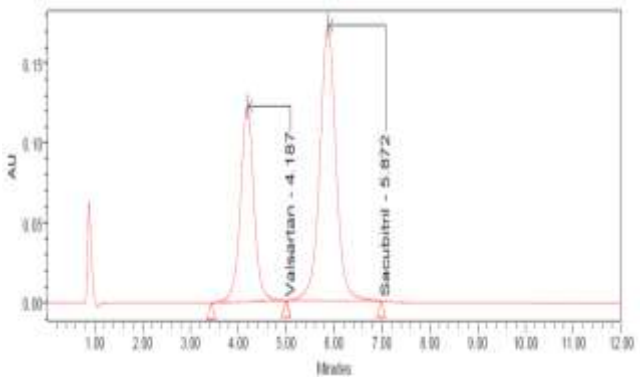


Fig :4 Sample

2. Accuracy (Recovery)

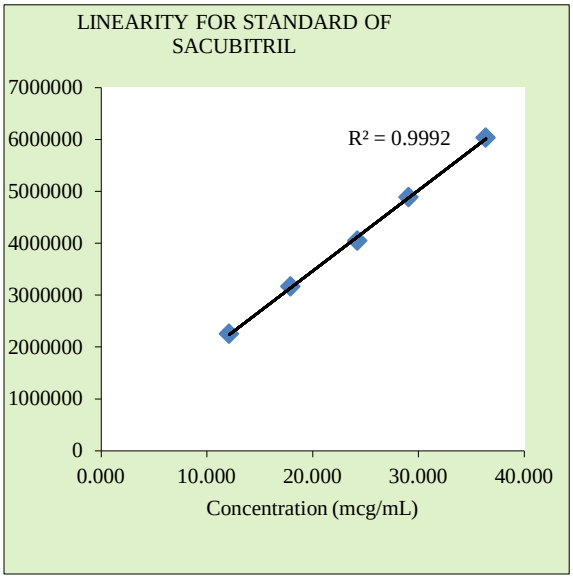
The degree to which the value found and the value recognised as either a conventional true value or a reference value agree with one another is expressed as an analytical procedure's accuracy.Accuracy of the analytical method was evaluated at a known concentration of Sacubitril and Valsartan at about 50%, 100% and 150% of test concentration of sample solution was calculated. % accuracy at individual level and overall average of % Recovery at all level for Sacubitril and Valsartan found to be in the range 99% to 101 % and %RSD for %assay of all levels found to be less than 2 % which has been depicted in the Table No.4

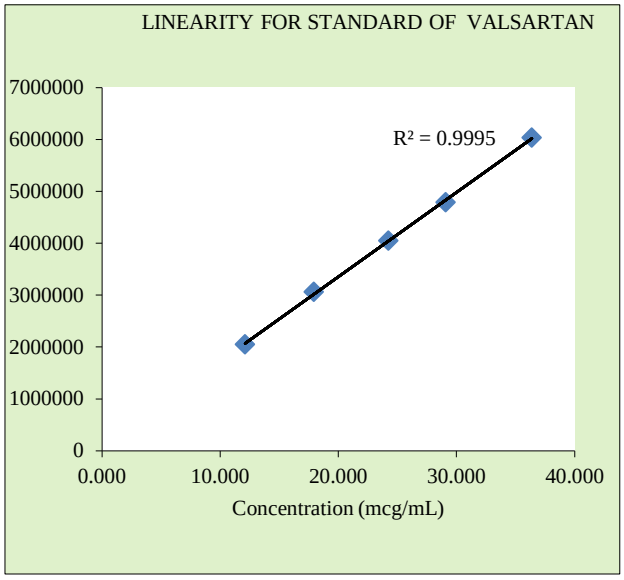
Table 4 Recovery Studies of Sacubitril and Valsartan

Level of Recovery	Drug	% Recovery
50%	SAC	99.9
	VAL	99.5
100%	SAC	100.3
	VAL	100.07
150%	SAC	100.06
	VAL	99.41

3. Linearity

For the evolution of the linearity of the analytical method, standard dilutions of Sacubitril and Valsartan in a concentration range of 50 µg/ml to 150 µg/ml for Sacubitril and Valsartan prepared as per the test procedure of methodology and analyzed on the HPLC system. In below results of linearity plot is mentioned in graphical representation . And linearity results shown in table 5





Graphical representation

4. Precision

When several samples of the same homogeneous material are taken under the specified conditions, the precision of an analytical process describes how closely the measurements agree (the degree of scatter) over time.

A.Method precision (Repeatability):

The precision can be determined by using sample with RP-HPLC method. Samples solution of six replicate injection containing Sacubitril and Valsartan 97/103 mg injected for determining the % RSD which less than 2.0%.

B. System precision:

The resolution between the peaks of Sacubitril and Valsartan should not less than 2.0 from system suitability solution. The tailing factor for Sacubitril and Valsartan peaks in the standard preparation should not be more than 2.0.The relative standard deviation for the average area of Sacubitril and Valsartan peak five replicate injections of standard preparation should not be more than 2.0%.

C. Intermediate precision:

% Assay of individual and average value should be 90.0% to 110.0% of labeled amount of Sacubitril and Valsartan.The %RSD for the assay results of six sample preparations should not be more than 2.0.The %RSD for the combined assay results of 12 replicate preparations between method precision and intermediate precision should not be more than 2.0%**.** And summary of precision results shown in table 5

5. Robustness :

The ability of an analytical procedure to be unaffected by deliberate, but slight, changes to method parameters is known as robustness, and it provides an estimate to how reliable the approach will be under typical conditions.

A. Flow rate (± 0.2 mL/min)

System suitability criteria should pass as per system suitability requirements in in all altered conditions. The % assay difference between altered conditions to initial condition should not be more than ± 2.0%.

B. Wavelength (±2nm)

System suitability criteria should pass as per system suitability requirements in in all altered conditions. The % assay difference between altered conditions to initial condition should not be more than ± 2.0%.All the known impurities are well separated from each other the Sacubitril & Valsartan peaks.

C. Column Temperature (± 5°C)

System suitability criteria should pass as per system suitability requirements in in all altered conditions.The % assay difference between altered conditions to initial condition should not be more than ± 2.0% and summary of results shown in table 5

6. Solution stability

Solutions shall be stored at room temperature and in refrigerator condition (2-8°C). Perform solution stability for standard and sample at Hour-4, Hours-12, etc and calculate % recovery for standard solution and sample solutions against initial tested solutions. In fig 5 24 hrs chromatograms is mentioned below. And summary report of solution stability shows in table 5.

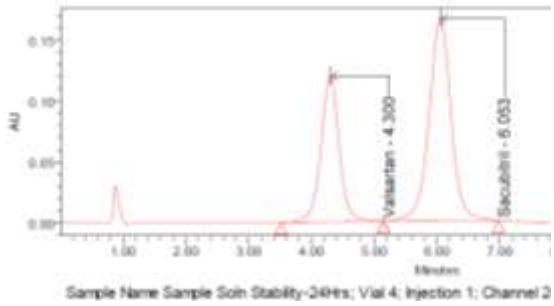


Fig 5 24 hrs solution stability sample

7. Filter validation:

Evaluate system suitability parameter using standard solutions. Analyze test samples as per Methodology given , evaluating the effect of the filtration on the sample solution using 0.45 µm nylon, against centrifugation at 3000rpm for 10 minutes**.** And the summary report of filter validation shown in table 5

Table 5 Result and Acceptance criteria for the validation of developed method.

Sno	Study	Result	Acceptance criteria
1	**System suitability**	The % RSD of five replicate injections of standard solution is 1.7% for Sacubitril and 1.3% for Valsartan.	The standard solution's five measurements' percent RSD should be NMT 2.0.
2	**Recovery**	Recovery is between 98.0% to 102.0%	The recovery should be 98.0% to

		with the acceptable RSD of NMT 2.0% at each level	102.0 % with the RSD of NMT 2.0% at each level.
3	Precision	RSD of % assay results of six-sample preparation is 1.1 for Sacubitril and 0.7 for Valsartan	% RSD for percentage assay results of six samples preparation should be NMT 2.0
4	**Linearity**	The correlation coefficient is **0.9992** for Sacubitril and **0.9995** for Valsartan.	It must be more than or equal to 0.999 for the correlation coefficient.
5	**Robustness**	At different deliberates changes in chromatograph conditions % RSD was NMT 2.0.	% RSD should be NMT 2.0.
6	**Solution stability**	The Cumulative % RSD of area for Sacubitril and Valsartan obtain from standard solution at different time intervals Solution Stability and Mobile Phase stability is 0.1	NMT 2.0
		Physical appearance of mobile phase is stable	Informative
7	**Filter Validation**	The results obtained by centrifugation, 0.45µm Nylon filter and 0.45µm PVDF (with discarding volumes of 1mL, 2mL and 3mL)	The % Difference between the average % assay value of the centrifuged preparations and the % assay value of each filtered preparation should not be more than ± 2.0%**.**

The goal of the current work is to quantify the combined dosage of sacubitril and valsartan using RP-HPLC as well as a UV detector. Based on Sacubitril & Valsartan, the physical and chemical parameters were selected. The stationary phase was selected based on the suitability of the system. Analytes were separated using Scientific Hypurity C18 (100 mm X 4.6 mm), 5 m, to assess the parameters. Mobile phase optimisation was carried out on various pilot studies. Tetrahydrofuran 500ml and a preparation of 0.1% orthophosphoric acid in 500ml of mobile phase. For the system suitability characteristics, the mobile phase ratio of solutions A and B was found to be an optimal combination of 65:35 (%v/v). For the purpose of evaluating the chromatographic peak resolution with the least amount of solvent consumption, various preparatory trials including the mobile phase ratio, flow rate, wavelength, and column temperature were conducted. The filter validation studies are a crucial component of the Qc testing. Samples were filtered using syringe filters, and the filtrate was then collected and put through an HPLC analysis to determine the amount of API. To verify 100% recovery and determine the analyte binding in a syringe filter, centrifuged samples were utilised as controls. The investigations on solution stability were also carried out by storing the sample and standard solution at the correct room temperature in order to assess their stability. The developed methodologies were validated in accordance with ICH principles, which were clear, concise, and dependable and were effectively employed for the quantification of Without any excipient intervention, sacubitril and valsartan.

CONCLUSIONS

The Indian Pharmacopeia, British Pharmacopeia, and United States of Pharmacopeia do not have any authorised methods for valsartan/sacubitril in bulk and in commercially accessible tablets with less retention time, accuracy, and sensitivity. Very delicate and exact analytical techniques are needed to accomplish these goals. There has been a lot of study in drug design, bioavailability, and safety that has been motivated by the

improvement of life quality. It's critical to develop an analytical method just for analysing a pharmaceutical compound in a specific matrix. The suggested approach outlines the creation of a sensitive, accurate, and selective RP-HPLC method for the simultaneous measurement of sacubitril and valsartan in pure and commercial formulations with good resolution. The validation outcomes demonstrated that the recommended RP-HPLC technique is suitable for the planned usage and can be quickly and easily applied for routine quality control of valsartan and sacubitril in combined dosage forms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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