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SIMULTANEOUS ESTIMATION OF VALSARTAN AND SACUBITRIL IN PURE AND MARKETED FORMULATION BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Valsartan and Sacubitril, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Sunfire C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (60:40% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 220nm. The retention time of the Sacubitril and Valsartan was 3.0, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Sacubitril and 75-375µg/ml of Valsartan. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Sacubitril, Valsartan, RP-HPLC, Validation.

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INTRODUCTION

Liquid chromatography is the most widely used analytical tool in the pharmaceutical industry and reversed-phase is the most frequently used mode. During the drug development process, liquid chromatographic methods are used to determine the quality of the drug substance (active pharmaceutical ingredient) and drug product Sacubitrilis chemically (S)-5-[(4-phenylphenyl)methyl] pyrrolidin-2-one belongs to the class of neprilysin inhibitor, used as anti-hypertensive. Molecular Formula –$C_{17}H_{17}NO$, Molecular Weight – 251.32 g/mol, Solubility - Slightly soluble in water, sparingly soluble in dehydrated alcohol, freely soluble in methanol. Valsartan is chemically [1-6] (2S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazole-5-yl)biphenyl-4-yl]methyl} pentanamido) butanoic acid. Valsartan is potent Angiotensin II receptor blocker. Itis mainly used as anti-hypertensive drug. Valsartan is official in IP and USP. The (S)enantiomer is essentially used. Molecular Formula –C24H29N5O3, Molecular weight- 435.5 g/mol and Soluble in Acetonitrile, practically in soluble in water also soluble in methanol.

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The aim of the present study was to develop accurate, precise and selective reverse phase HPLC assay procedure for the analysis of Sacubitril and Valsartan in synthetic mixture.

From the literature survey it was found that many methods are available for determination of Valsartan individually and few methods in combination with other drugs. However, few [4-15] HPLC method were reported for simultaneous determination of Sacubitril and Valsartan in combination. In the proposed study an attempt will be made to develop a HPLC method for simultaneous estimation of Sacubitril and Valsartan. Pharmaceutical grade of Sacubitril and Valsartan were kindly supplied as gift samples by Manus Akketeva, Ahmadabad, India and Lupin Ltd respectively,

certified to contain > 99% (w/w) on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Chemicals, Ran Kem, India. The validation of proposed method is done according to the ICH [15,16] guideline validation was done according to ICH guidelines

AIM AND OBJECTIVES

Review of literature for Valsartan and Sacubitril gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs. Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Valsartan and Sacubitril and single method is available for such estimation by RP-HPLC. In view of the need for a suitable RP-HPLC method for routine analysis of Ranitidine and Dicyclomine in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Valsartan and Sacubitril and extend it for their determination in formulation. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.

The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

Optimization of Column:

The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Sunfire C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (40:60v/v)
Flow rate : 0.9ml/min
Wavelength : 220nm
Injection volume : 10 µl
Run time : 6min

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Scubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Scubitril and 2.25ml of the Valsartan stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Scubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Scubitril and 2.25ml of the Valsartan stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Scubitril and Valsartan sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 2.25ml of the Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

MATERIALS AND METHODS

METHOD DEVELOPMENT

Equipment:

Chromatographic separation was performed on WATERS Alliance 2695 separation module, Software, consisting of Empower 2, 996 PDA Detector and

injector with 10µl loop volume. LC solution software was applied for data collecting and processing.

Reagents and chemicals:

Acetonitrile and methanol of HPLC grade were procured from Merk lab ltd. Sacubitril and Valsartan standards were received as gift samples from Manus Akketeva and Lupin Ltd, India, respectively

Selection of detection wavelength:

The standard solution of Sacubitril (10 µg/ml) and Valsartan (10 µg/ml) in methanol was individually scanned over the range of 200 nm-400 nm. Its overlay graph showed that both the drug absorb at 220 nm (As show in figure- 3). So, the wavelength selected for the determination of Sacubitril and Valsartan was 220 nm.

HPLC Conditions:

A SheisedoC18 (250*4.6 mm, 5µm) column was used as the stationary phase. A mixture of Acetonitrile and water in the ratio of (40: 60 %v/v). It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 267nm.The injection volumes of sample and standard were 10µl.

Standard solutions:

A stock solution containing 1000µg/ml of Sacubitril and Valsartan were prepared separately by dissolving in methanol. A working standard solution containing 50-250 µg/ml and 50-250 µg/ml of Sacubitril and Valsartan were prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

ASSAY OF TABLET FORMULATION

To determine the content of Sacubitril and Valsartan simultaneously in conventional tablet (ENTRESTO, label claim 24 mg Sacubitril 26 mg Valsartan); twenty tablets were accurately weighed, average weight was determined and grounded to fine powder.

A quantity of powder equivalent to 24 mg Sacubitril and 26 mg Valsartan was transferred into 100 ml volumetric flask containing 50 ml Methanol, sonicated for 10 min. and diluted to mark with same solvent to obtain 20 µg/ml Sacubitril and 175 µg/ml Valsartan.

The resulting solution was filtered using Whatman filter paper. From the above solution 3 ml was transferred into 10 ml volumetric flask and diluted to mark with same solvent. So, Resultant solution was found to contain 20 µg/ml Sacubitril and 75 µg/ml Valsartan.

This Test solution was injected and chromatogram was recorded for the same. The amount of drugs were calculated and the results are given in Table 3.

METHOD VALIDATION

The developed method was validated as per ICH guidelines for its System suitability, linearity, accuracy, precision, robustness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in Table 5.

System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factors were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Sacubitril and Valsartan at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between peak area Vs concentration of the drug. The responses were found to be linear in the range 5-20 µg/ml and 75-325 µg/ml for Sacubitril and Valsartan.

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (50%, 100% and 150%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Precision

Repeatability

Standard solutions of 5, 10, 15 and 20 µg/ml Sacubitril and 75, 150, 225,300 and 325µg/ml Valsartan were prepared and Chromatogram were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

Intraday precision

Mixed solutions containing 10, 15, 20 µg/ml of Sacubitril and Valsartan were analyzed three times on the same day and % R.S.D was calculated.

Interday precision

Mixed solutions containing 10, 15, 20 µg/ml of Sacubitril and Valsartan were analyzed three times on different days and % R.S.D. was calculated.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average

slope and standard deviation from the calibration curve as per ICH guidelines.

Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of both drugs was noted. The factors selected were flow rate, pH of the mobile phase and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters.

RESULTS AND DISCUSSION

The present manuscript deals with simultaneous estimation of SAC and VAL in combined tablet dosage form by RP- HPLC method using mobile phase as the solvent. The developed method is based upon estimation of both the drugs by determining the area under curve of the chromatogram at selected analytical wavelength. The linearity of the proposed method was established by least square regression analysis of the calibration curve The drug response was linear (R2 = 0.9999 for SAC and 0.9999 for VAL) over the concentration ranges between 20-320 µg/ml for both SAC and VAL as shown in the Table.

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 50%, 100% and 150% of the labeled amount of both the drugs (80 mg SAC and VAL each) in tablet formulation as shown in Table 1.

Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3) and mean % recovery (n = 3) were determined and summarized in Table 1 and 2. Intra-day precision was estimated by assaying samples of the tablet formulation containing 80 µg/ml of SAC and VAL each, six times and the results were averaged for statistical evaluation. The statistical validation data for intra-day precision is summarized in Table 3 & 4.

Inter-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 80µg/ml of SAC and VAL each, three levels analysed on three consecutive days. The statistical validation data for inter-day precision is summarized in Table 5. Both intra-day and inter-day variation showed less % RSD value indicating high grade of precision of the method as shown in table 6.

The Robustness was evaluated by analyzing the samples by varying few parameters like wavelength and flow rate. The statistical validation data is summarized in table 7 and 8.

The results of the methods lie within the prescribed limit, showing that method is free from interference from excipients The validation results obtained confirm the suitability of the proposed stability indicating RP-HPLC method for simple, accurate and precise analysis of SAC and VAL in pharmaceutical preparations. The proposed method does not need prior separation of SAC and VAL before analysis. In addition, it is suitable for application without interference of excipients and can be applied directly to the commercial preparation without previous treatment.

Table 1. Linearity data

Concentration µg/ml		Peak Area	
Valsartan	**Sacubitril**	**Valsartan**	**Sacubitril**
10	75	1215225	230247
15	150	2135937	462332
20	225	3020839	659905
25	300	4078841	892989
	375	5058145	1101075

Table 2. The accuracy results for Valsartan

%Concentration (at specification Level)	Peak Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	112.5	112.3	99.7%	99%
100%	420697.7	225	224.7	99%	
150%	631550.7	337.5	337.4	99%	

Table No. 3. The accuracy results for Sacubitril

%Concentration (at specification Level)	Peak Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	331938	7.5	7.3	99.88	100.166
100%	658274	15	14.7	98.89	
150%	970963	22.5	22.2	101	

Table 4. Results of Intermediate precision for Valsartan

Name of drug	RT	Area (µV*sec)	USP Plate count	USP Tailing
Valsartan	3.853	3075833	7039	1.1
Valsartan	3.857	3029583	9857	1.2
Valsartan	3.854	3021991	7881	1.1
Valsartan	3.855	3022485	7902	1.2
Valsartan	3.854	3085833	9285	1.1
Valsartan	3.853	3019482	8955	1.2

Table 5. Results of Intermediate precision Day 2 for Sacubitril

S.No	Peak Name	RT	Area (µV*sec)	USP Plate count	USP Tailing
1	Sacubitril	3.006	648822	6983	1.1
2	Sacubitril	3.008	640863	7728	1.2
3	Sacubitril	3.008	643382	9576	1.1
4	Sacubitril	3.007	641884	8275	1.2
5	Sacubitril	3.007	647822	9837	1.1
6	Sacubitril	3.005	649181	8744	1.2
Mean			645325.7		
Std. Dev.			3711.009		

Table 6. Results for Robustness-Valsartan

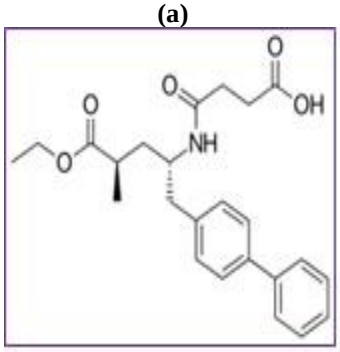
Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

Table 7. Results for Robustness -Sacubitril

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

Fig.1.Chemical structures of (a) Sacubitril and (b) Sacubitril.

(a)



(b)

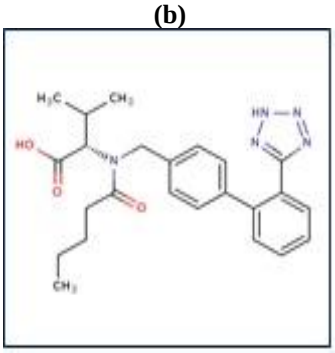


Fig. 2. Typical chromatogram of Valsartan and Sacubitril

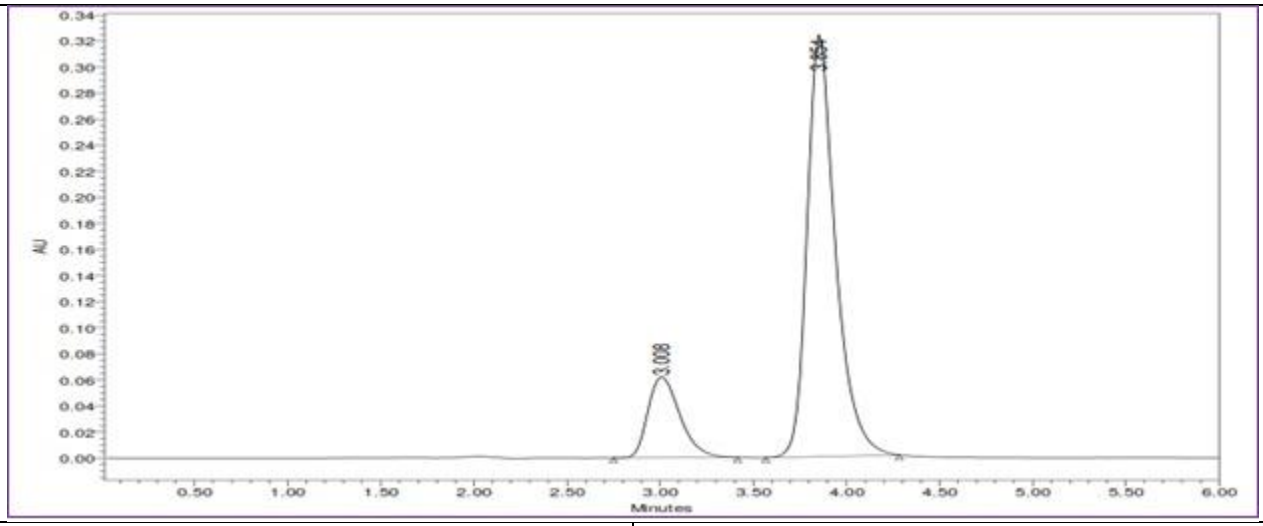


Fig.3. Calibration curve of sacubitril

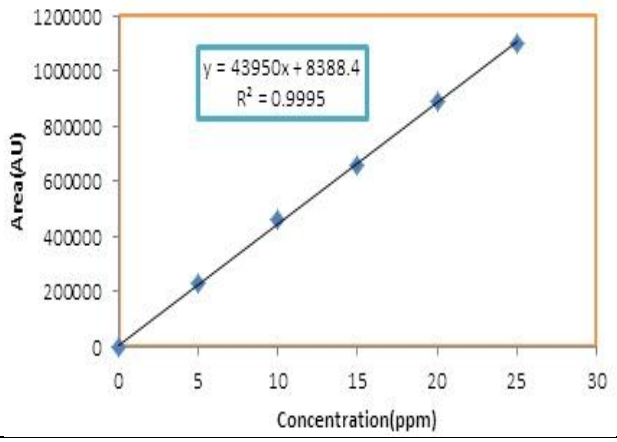
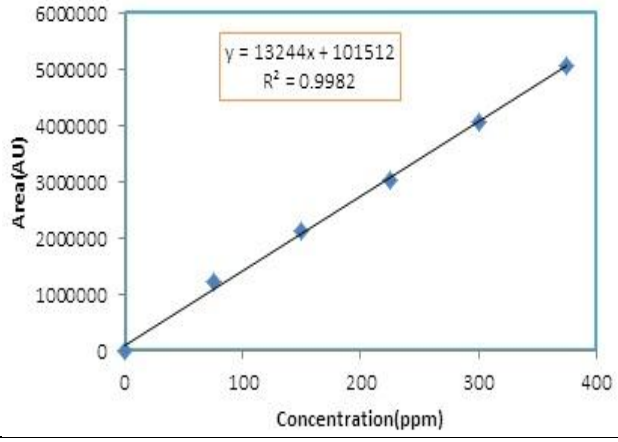


Fig.4. Calibration curve of Valsartan



SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 220nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Sunfire C18 (4.6×250mm) 5µ because it was giving good peak. 35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.9ml/min because of good peak area and satisfactory retention time. Mobile phase is Water and Acetonitrile (60:40% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 6min because analyze gave peak around 3.0, 3.8 ±0.02min of Sacubitril and Valsartan respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 5-25µg/ml of Sacubitril and 75-375 µg/ml of Valsartan of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Valsartan and Sacubitril in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Valsartan and Sacubitril was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile (60:40% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Valsartan and Sacubitril in

bulk drug and in Pharmaceutical dosage forms.

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