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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the simultaneous estimation of sacubitril and valsartan in tablet dosage forms. Chromatographic separation was achieved on a Inertsil ODS 3V column using mobile phase consisting of a mixture of 50 volumes of Triethylamine buffer, and 50 volumes of acetonitrile; pH 3.5 at isocratic mode and eluents were monitored at 239nm.With the optimized method, the retention times of sacubitril and valsartan were found to be 2.523 and 4.410mins respectively with theoretical plate count and assymetry as per the ICH limits. Linearity was observed in the concentration range of 5-15µg/ml for Sacubitril (r^2 =0.999) and 2.5-7.5 µg /ml for Valsartan (r^2 =0.999). The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. The percentage assays were found to be 100.27% and 101.98% respectively. Limit of detection and Limit of quantification values were found to be 0.015, 0.038 and 0.004,0.011 respectively. All statistical data proves validity of the method in accordance with ICH guidelines and hence can be successfully applied to the simultaneous estimation of Sacubitril and Valsartan.

KEYWORDS: Sacubitril, Valsartan, RP-HPLC, ICH Guidelines.

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.[1-4] Methods such as HPLC[5-7], LC-MS[8-9], protein precipitation[10] and simultaneous UV-spectrophotometric methods[11-12] 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.[13]

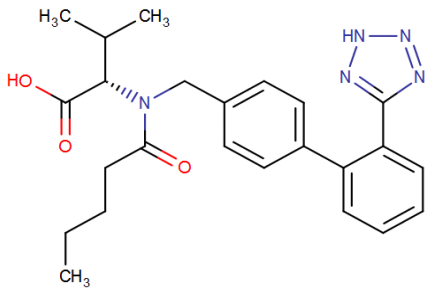

Figure 1: Chemical structure of valsartan.

Sacubitril is an antihypertensive drug used in combination with valsartan for the treatment of heart failure.[14-15] Literature search reveals that only two analytical methods were reported for simultaneous estimation of sacubitril and valsartan from rat plasma using LC-MS/MS[16] and from a synthetic mixture using HPLC.[17]

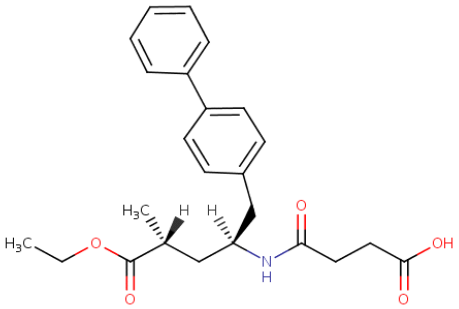

Figure 2: Chemical structure of valsartan.

There is no stability indicating analytical methods were reported for simultaneous estimation of sacubitril and valsartan. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for the

simultaneous estimation of sacubitril and valsartan from API and pharmaceutical dosage form.

MATERIALS AND METHODS[17]

Materials and reagents

Triethyl amine, Acetonitrile, orthophosphoric acid HPLC and AR grade were procured from Merck and Rankem lab ltd. Sacubitril and valsartan standards were received as gift samples from KP labs, Hyderabad, India and the combination collected from local pharmacy.

Instrumentation

Chromatographic separation was performed on HPLC system consist of model Shimadzu LC-20 AT having SPD-20AT detector and rheodyne injector with 20µl loop volume. Spinchrom software was applied for data collecting and processing. UV spectrophotometer which consists of model Systronic 119 is also used to measure the wavelength of the solution of Sacubitril and Valsartan.

Preparation of mixed standard solution

Weigh accurately 10mg of SACUBITRIL and 15 mg of VALSARTAN in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 µg/ml of Sacubitril and 150 µg/ml of Valsartan is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets were weighed (each tablet Entresto containing sacubitril-24mg and valsartan-26mg and taken into a mortar and crushed to fine powder and uniformly mixed taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of valsartan and sacubitril (µg/ml) were prepared by dissolving weight equivalent to 10 mg of sacubitrl and 15mg of valsartan and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of sacubitril and 150µg/mlof valsartan was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Chromatographic conditions

Inertsil ODS C18 (250*4.6 mm, 5µm) column was used as the stationary phase. A mixture of triethylamine (pH 3.0) and acetontrile in the ratio of (50:50 %v/v) was used as a mobile phase and pH 3.0 adjusted with ortho phosphoric acid. It was filtered through 0.45µ (micron) membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 239nm. The injection volumes of sample and standard were 20µl (microliter). Total run time is 6mins.

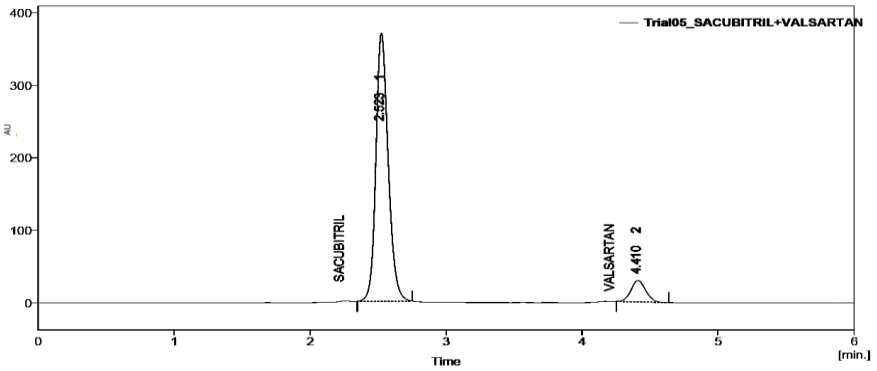

Figure 3: optimised chromatogram.

The developed Method was validated for linearity, precision, accuracy, robustness and is applied for forced degradation studies as per the ICH guidelines.[18-23]

Assay

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 10mg of SACUBITRIL and 15mg of VALSARTAN in 100ml volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 µg/ml of SACUBITRIL and 150 µg/ml of VALSARTAN is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

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$$\%\text{Assay of Drug} = \frac{\text{Calculated or Sample Weight}}{\text{Sx Label claim}}$$

RESULTS AND DISCUSSION

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factor was calculated. The results are given in table 1.

Table 1: Optimized method parameters.

S.No	Name	Retention time	Peak area	Resolution	Tailing factor
1	Sacubitril	2.523	2325117	-	1.40
2	Valsartan	4.410	2241282	10.248	1.19

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. The calibration curve showed (Fig.4 and 5) good linearity in the range of 2.5-7.5 µg/ml, for sacubitril with correlation coefficient (r2) of 0.999 and 5-15 µg/ml for valsartan with correlation coefficient (r2) of 0.999. Results are given in table -2.

Table 2: Linearity.

S.No	Concentration (µg/ml,)	Area
sacubitril	5	2240.61
	7.5	3307.13
	10	4521.12
	12.5	5566.07
	15	6772.74
valsartan	2.5	1878.12
	3.75	2773.21
	5	3791.21
	6.25	4668.21
	7.5	5676.52

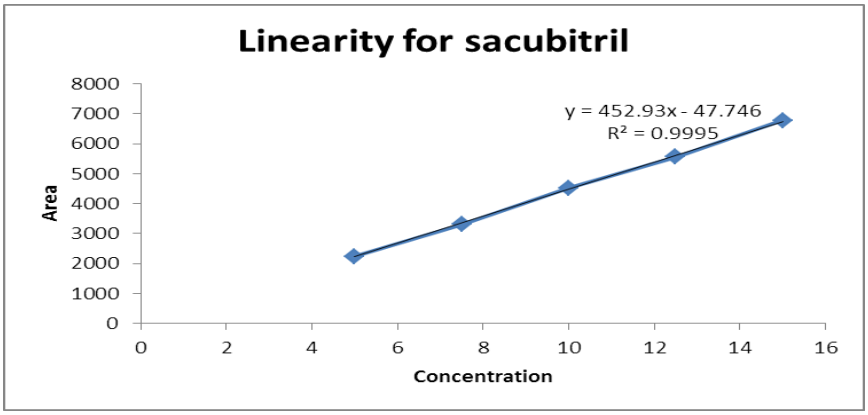

Figure 4: Linearity graph of sacubitril.

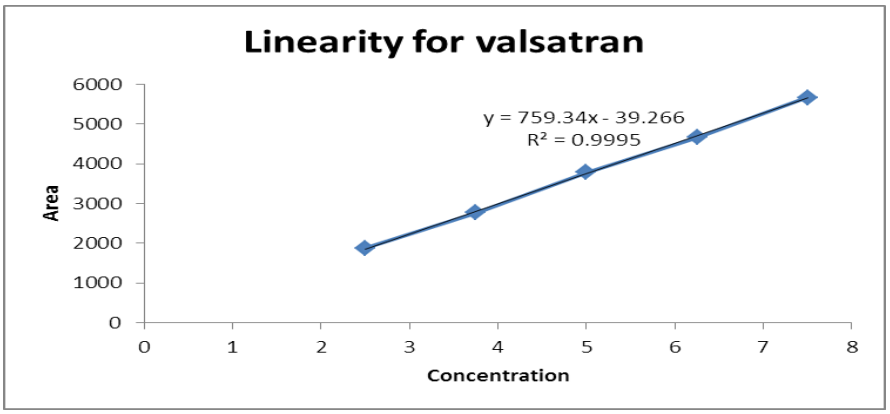

Figure 5: Linearity graph of valsartan.

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. At each concentration, sample was injected thrice to check repeatability and from the % RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.72-100.02% for the Sacubitril and 99.87- 100.17 % for valsartan at three different concentrations 80%, 100%, 120%. The results are given in table 3 and 4.

Table 3: Accuracy table of sacubitril.

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	5	4.0021	96.27	99.61
	5	4.0901	96.33	
	5	4.0211	96.45	
100%	10	91230	101.86	
	10	9.1170	101.67	
	10	9.320	101.80	
150%	15	13.1231	100.72	
	15	13.1342	100.65	
	15	13.1424	100.69	

Table 4: Accuracy table of Valsartan.

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	2.5	1.5300	100.21	100.58
	2.5	1.4309	100.56	
	2.5	1.5670	100.45	
100%	5	3.0010	100.24	
	5	3.0230	100.87	
	5	4.0120	100.86	
150%	7.5	6.5661	100.43	
	7.5	6.5632	100.31	
	7.5	6.5564	10.23	

Precision

Method Precision

Standard solution containing sacubitril (24.5 µg/ml) and valsartan (25.5 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated. The results are givenin table 5.

Table 5: Method Precision.

Intraday data of Sacubitril			Interday data of Valsartan		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.507	2350119	1	4.397	202888
2	2.497	2341355	2	4.390	208551
3	2.507	2319879	3	4.397	208704
4	2.507	2305220	4	4.397	200953
5	2.523	2321138	5	4.390	205011
6	2.523	2331562	6	4.413	201450
Avg		23122.21	Avg		209.98
SD		1.32	SD		1.32
%RSD		0.58	%RSD		0.62

System precision of sacubitril and valsatran

Table 6: system precision of sacubitril and valsartan.

Intraday data of Sacubitril			Interday data of Valsartan		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	2.510	2192247	1	4.397	227545
2	2.523	2322573	2	4.410	211442
3	2.523	2321138	3	4.413	202102
4	2.523	2333196	4	4.413	200853
5	2.507	2350119	5	4.397	202888
6	2.497	2341315	6	4.390	228551
Avg		2314.67	**Avg**		227.80
SD		1.25	**SD**		1.39
%RSD		**0.56**	**%RSD**		**0.61**

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

- Based on Signal-to-Noise.
- Based on the Standard Deviation of the Response and the slope.

$LOD = (3.3 * \sigma) / S$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. From the linearity data calculate the limit of detection and quantitation, using the following formula,

$LOQ = (10 * \sigma) / S$

When,σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table 7: LOD & LOQ results.

S.No	Drug name	LOD (µg/ml)	LOQ (µg/ml)
1	Sacubitril	0.015	0.038
2	Valsartan	0.004	0.011

Robustness

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (+ 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in factor of (% RSD< 2%) the developed RPHPLC method for the analysis of sacubitril and valsartan. The results are given in table 8 and 9.

Table 8: Robustness.

S.no	Robustness parameter		Sacubitril		Valsatran	
			Retention time	**Tailing factor**	**Retention time**	**Tailing factor**
	Flow rate	0.8 ml	3.130	1.258	5.443	1.167
		1.02ml	2.090	1.036	3.663	0.943
	Wave length	237 nm	2.513	1.222	4.380	1.088
		241 nm	2.517	1.179	4.380	1.125
	pH	3.1	2.190	1.136	3.653	0.953
		37	2.617	1.189	4.320	1.135

Assay

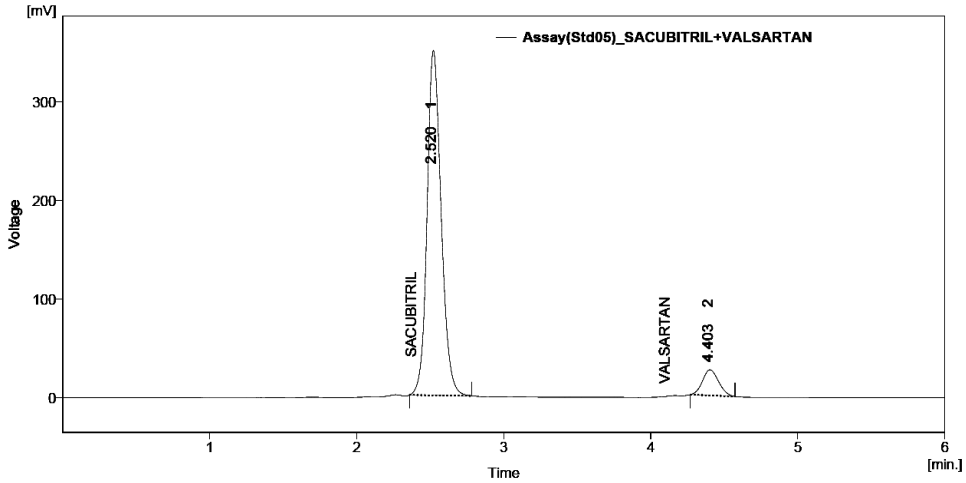

Figure 6: ASSAY Chromatogram.

Table 9: Assay Results.

	SACUBITRIL		VALSARTAN	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2334362	2344463	207967	212684
Injection-2	2323199	2351614	199968	209655
Injection-3	2337863	2337863	207039	207039
Injection-4	2331502	2334732	207632	210092
Injection-5	2328483	2341801	198197	210080
Average Area	2331082	2342095	2041606	209910
Standard deviation	6.48		6.45	
%RSD	0.27		0.30	
Assay(%purity)	**100.27**		**101.98**	

Table 10: Summary.

Parameter	Sacubitril	Valsartan	LIMIT
Linearity			
Range	5-15µg/ml	2.5-7.5µg/ml	R < 1
Regression coefficient	0.999	0.999	R < 1
Slope(m)	205.4	30.36	R < 1
Intercept(c)	5.604	22.47	R < 1
Regression equation (Y=mx+c)	Y=205.4x+5.604	Y=30.36x+22.47	
Specificity	Specific	Specific	No interference of any peak
System precision % RSD	0.56	0.61	NMT 2.0%
Method precision % RSD	0.52	0.62	
Accuracy	99.61	100.58	98-102%
LOD	0.015	0.038	NMT 3
LOQ	0.004	0.011	NMT 10
Assay	100.27	101.98	98-102%
Robustness	0.8-1.2%	0.8-1.2%	
Retention time	2.523 mins	4.410 mins	

CONCLUSION

This newly developed method for the simultaneous estimation of Sacubitril and Valsartan was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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