WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

RP-HPLC Method for the Simultaneous Estimation of Sacubitril and Valsartan in Bulk and Tablet Dosage Form

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ABSTRACT

The development and validation for the simultaneous estimation of Sacubitril and Valsartan achieved by X-Terra RP-18 (150mm*4.6mm, 5µm) column, with a mobile phase of 0.1% formic acid in water and Methanol (25:75% v/v at a flow rate of 0.8ml/min at ambient temperature. The peaks were observed by a UV-detector at 267nm and the retention time for both the drugs was 3.156 and 2.663min. The percentage recovery was 99-100% for both the drugs. Method validation was carried out for all above new methods developed. Linearity parameter for the method was analysed by preparing solution of five levels of concentrations. Calibration curve for concentration versus peak areas was found linear. Precision was studied by preparing solutions in six replicates and analyzed, % RSD was calculated for the peak areas of each drug. Accuracy for new methods developed, evaluated by using the standard solutions of three different concentrations (50%, 100% and 150%) into sample solution and their percentage recovery at each level was determined. Robustness for above methods was carried out at different flow rates and at different organic composition, standard and sample solutions were prepared and analyzed at ± 0.1ml/min and at ± 10% and developed method found to be stable on deliberate variation. Parameters evaluated for validating novel RP-HPLC methods for simultaneous determination of above dosage forms meet the requirements of ICH guidelines. The developed methods are simple, precise, cost effective and rapid. These newly developed methods can be applied for the quantitative determination in the QC laboratories for the regular release

.Keywords: Sacubitril; Valsartan; RP-HPLC; Method development; Validation

METHOD DEVELOPMENT AND OPTIMIZATION OF METHOD

Preparation of Buffer solution

1ml of formic acid was taken into a clean in 1000ml beaker and dissolved in HPLC grade water and the volume was adjusted to 1000ml. The resulting solution was sonicated for 15min. Then the solution was filtered through 0.45µl nylon filter.

Preparation of mobile phase

The mobile phase was prepared by taking 25 volumes of buffer and 75 volumes of methanol mixed well and sonicated for 15 min. Then the solution was filtered through 0.45µl nylon filter.

Diluent

Water: Acetonitrile (50:50% v/v).

Preparation of standard solution

A 48.5 µg of pure Valsartan and 51.5 µg of Sacubitril were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 97µg/ml of valsartan and 103 µg/ml sacubitril.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 970µg of valsartan and 1030µg of sacubitril sample were weighed and transferred to 10 ml of volumetric flask and dissolved in diluent. The flask volume was made up to mark with diluent and was shaken to give a solution containing 97µg/ml of valsartan and 103 µg/ml of sacubitril.

Determination of Working Wavelength (λ max)

10 mg of the Valsartan and Sacubitril standard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml Volumetric flask and made up to the mark with the water to give a concentration of 10µg/ml. The above prepared solution is scanned in UV between 200-400 nm using water as blank. The λ max was found to be 267nm.
After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (25:75%v/v). The flow rate was 0.8ml/ minute brought sharp peaks. The chromatogram was shown in Results & Discussion.

Trial-1

Chromatographic Conditions

Buffer	: 0.01 M Ammonium acetate in Water Mobile
Phase	: Buffer: Methanol (40:60% v/v)
Column	: Inertsil ODS 3V column (150*4.6mm, 5µm)
Flow Rate	: 0.8ml/min
Temperature	:
Ambient Injection Volume	: 10µl
Detector	: 267nm
Diluents	: Water: Acetonitrile (50:50)
Run time	: 10 Min

Trial-2 Chromatographic Conditions

Buffer	: 0.1% Formic acid in Water
Mobile Phase	: Buffer: Acetonitrile (50:50%v/v)
Column	: X-Terra-RP18 column (150*4.6mm, 5µm)
Flow Rate	: 0.8ml/min
Temperature	:
Ambient Injection Volume	: 10µl
Detector	: 267nm
Diluent	: Water: Acetonitrile (50:50%v/v**)**
Run time	: 10 Min

Trial-3 Chromatographic Conditions

Buffer	: 0.1% Formic acid in water
Mobile Phase	: Buffer: Methanol (25:75%v/v)
Column	: X- Terra RP-18column (150*4.6mm, 5µm)
Flow Rate	: 0.8ml/min
Temperature	: Ambient

Injection Volume	: 10µl
Detector	: 267nm
Diluent	: Water: Acetonitrile (50:50%v/v)
Run time	: 10 Min

Optimization Method for Simultaneous Estimation of Sacubitril and Valsartan in Bulk and Tablet Dosage Form by RP-HPLC

Chromatographic Conditions

Buffer	: 0.1% Formic acid in water Mobile
Phase	: Buffer: Methanol 25:75%v/v)
Column	: X- Terra RP-18column (150*4.6mm, 5µm)
Flow Rate	: 0.8ml/min
Temperature	: Ambient
Injection Volume	: 10µl
Detector	: 267nm
Diluent	: Water: Acetonitrile (50:50%v/v)
Run time	: 10 Min

ANALYTICAL METHOD VALIDATION

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50% - 150% for valsartan and 50%- 150% of sacubitril. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 48.5 µg /mL, 72.75µg/mL, 97µg/mL, 121.25µg/mL and 145.5µg/mL of Valsartan and 51.5g/mL, 77.25µg/mL, 103µg/mL, 128.75µg/mL and 154.5µg/mL Sacubitril.

Accuracy

The accuracy of the method was determined by calculating the recoveries of Valsartan and Sacubitril by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of valsartan and sacubitril.

Method Precision (Repeatability)

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of the method was checked by repeated preparation (n=6) of 97µg/ml of valsartan and 103µg/ml sacubitril without changing the parameter of the proposed chromatographic method and measured the peak areas and retention times.

Detection Limit

Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as: DL = 3.3 σ/ 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. The estimate of σ may be carried out in a variety of ways.

Quantification Limit

Based on the Standard Deviation of the Response and the Slope

The quantification limit (QL) may be expressed as: QL = 10 σ/ 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. The estimate of σ may be carried out in a variety of ways.

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

Ruggedness (Intermediate Precision)

The Method is said to be rugged when the results produced after analysis performed by another analyst are similar to the precision data of prior or another analyst performed. The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts.

System Suitability

The system suitability parameters such as US tailing factor, US theoretical Plates and resolution was achieved by injecting the prepared solution five times individually into the chromatographic system separately.

RESULTS AND DISCUSSIONS

METHOD DEVELOPMENT

Determination of Working Wavelength (λ max)

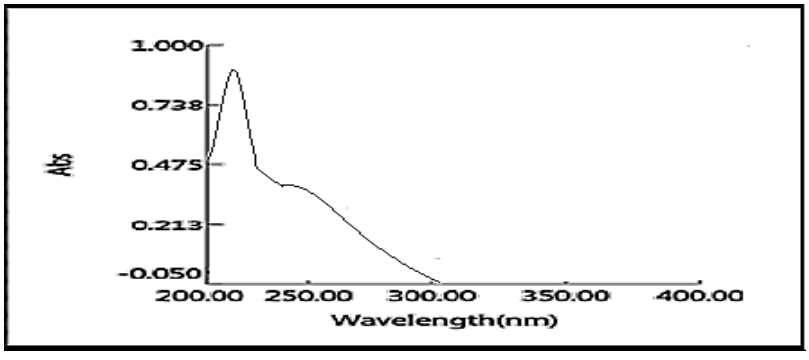

Fig No: 1 UV spectrum of standard Valsartan

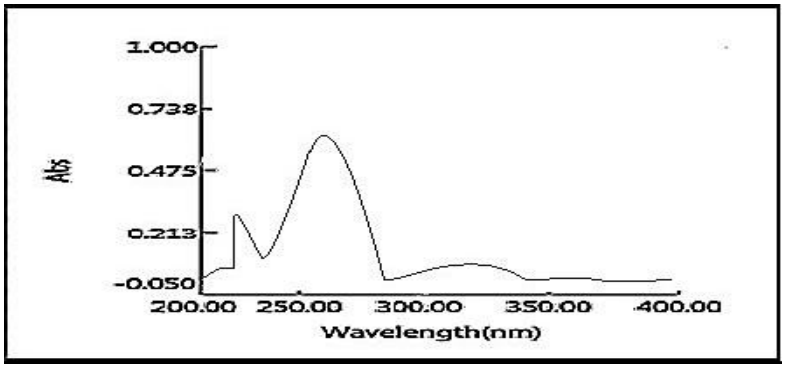

Fig No: 2 UV spectrum of standard Sacubitril

OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

Chromatographic Conditions

Buffer	: 0.1% Formic acid in water
Mobile Phase	: Buffer: Methanol (25:75%v/v)
Column Flow Rate	: X- Terra RP-18column (150*4.6mm,5µm)
Temperature	: 0.8ml/min
Injection Volume	: Ambient
Detector	: 10µl
Diluent	: 267nm
Run time	: Water: Acetonitrile (50:50%v/v)
	: 10 Min

Specificity

The chromatograms of standard and sample are identical with nearly same retention time. No interference due to placebo and sample at the retention time of analyte which shows that the method was specific. The results were given in the Table No 1

Table No: 1 Specificity data for Sacubitril and Valsartan

S.No	Sample Name	RT(min) Sacubitril	RT(min) Valsartan
1	Standard	3.159	2.664
2	Sample	3.154	2.665
3	Blank	-	-
4	Placebo	-	-

Result:

Chromatograms explain that retention time for standard, sample and commercial product of Valsartan and Sacubitril are same. This proves that, recipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

Linearity

Calibration curve with concentration verses peak areas was plotted by injecting the prepared solutions **(Fig No: 3 & 4)** and the obtained data were subjected to regression analysis using the least squares method and the data was represented in the **Table No: 2 & 3**

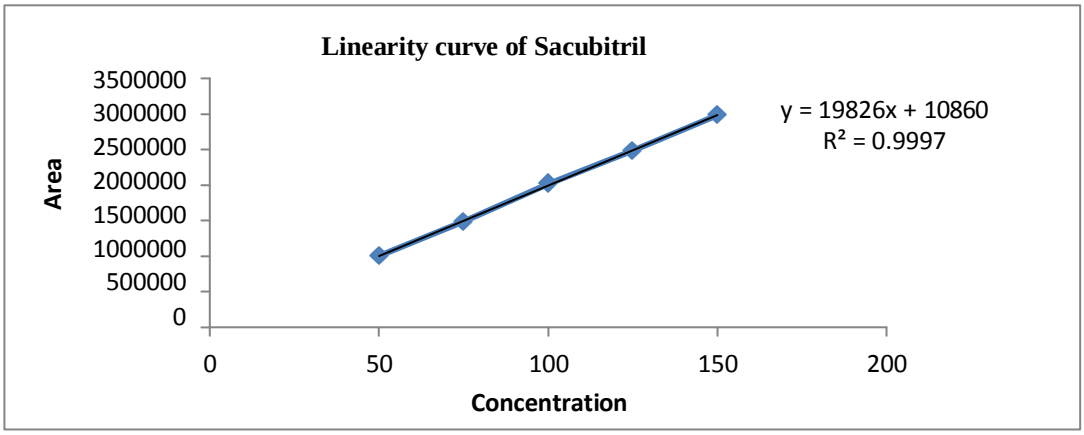

Fig No: 3 Linearity (calibration) curve of Sacubitril

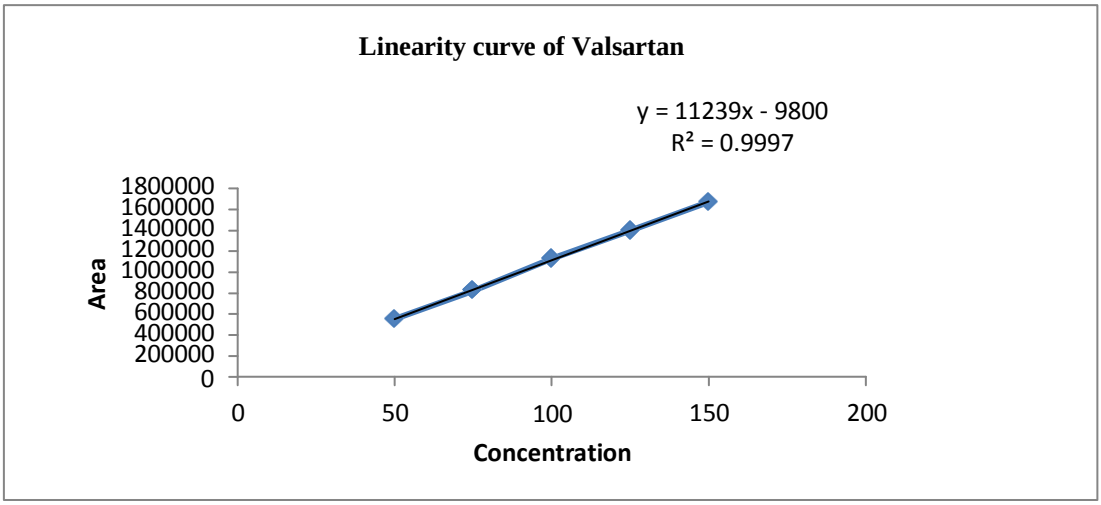

Fig No: 4 Linearity (calibration) curve of Valsartan

Table No: 2 Linearity data of sacubitril

S.NO	Level (%)	Sacubitril
		Area
1.	**50**	1002639
2.	**75**	1484258
3.	**100**	2016677
4.	**125**	2481096
5.	**150**	2982416
Correlation coefficient(r^2)		**0.9997**

Table No: 3 Linearity data of valsartan

S.NO	Level (%)	Valsartan
		Area
1.	**50**	552732
2.	**75**	824098
3.	**100**	1125464
4.	**125**	1396830
5.	**150**	1671196
Correlation coefficient(r^2)		**0.9997**

Result

A linear relationship between peak areas versus concentrations was observed for Valsartan and Sacubitril in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9997 and 0.9997 for both valsartan and sacubitril which prove that the method is linear in the range of 50% to 150%.

Table No: 4 Recovery data of Sacubitril and Valsartan

LEVEL	S.No	%Recovery of Sacubitril	Average of Sacubitril	%Recovery of Valsartan	Average of valsartan
50%	1	99.4	99.8%	99.7	99.4%
	2	99.7		99.2	
	3	100.4		99.3	
100%	1	99.8	99.6%	99.8	99.40%
	2	99.5		99.6	
	3	99.6		99.3	
150%	1	99.9	99.2%	99.3	99.1%
	2	99.1		98.9	
	3	98.7		99.1	

Result

Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate.

Method Precision (Repeatability)

Table No: 5 Summary of peak areas for method precision of sacubitril and Valsartan

S.No	Sacubitril			Valsartan		
	RT (min)	Area	%Assay	RT (min)	Area	%Assay
Injection1	3.154	2009107	99.8	2.665	1122986	99.8
Injection2	3.154	2001749	99.5	2.665	1125239	99.6
Injection3	3.155	2007748	99.6	2.665	1126274	99.3
Injection4	3.153	1998408	98.7	2.664	1125675	99.2
Injection5	3.153	2006631	99.1	2.665	1125561	98.4
Injection6	3.154	2004085	99.6	2.666	1124930	99.4
Mean	3.154	2004621	99.4	2.665	1125111	99.3
STDEV	0.00	4026.90	0.41	0.00	1134.72	0.48
% RSD	0.02	0.20	0.41	0.02	0.10	0.49

Result

Results of variability were summarized in the above **Table No: 5** Percentage Relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise**.**

Detection Limit

Table No: 6 LOD Values of valsartan and sacubitril

Parameter	**Valsartan (µg)**	**Sacubitril (µg)**
LOD	0.004	0.004

Method Precision (Repeatability)

Table No: 5 Summary of peak areas for method precision of sacubitril and Valsartan

S.No	**Sacubitril**			**Valsartan**		
	RT (min)	**Area**	**%Assay**	**RT (min)**	**Area**	**%Assay**
Injection1	3.154	2009107	99.8	2.665	1122986	99.8
Injection2	3.154	2001749	99.5	2.665	1125239	99.6
Injection3	3.155	2007748	99.6	2.665	1126274	99.3
Injection4	3.153	1998408	98.7	2.664	1125675	99.2
Injection5	3.153	2006631	99.1	2.665	1125561	98.4
Injection6	3.154	2004085	99.6	2.666	1124930	99.4
Mean	3.154	2004621	99.4	2.665	1125111	99.3
STDEV	0.00	4026.90	0.41	0.00	1134.72	0.48
% RSD	0.02	0.20	0.41	0.02	0.10	0.49

Result

Results of variability were summarized in the above **Table No: 5** Percentage Relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise**.**

Detection Limit

Table No: 6 LOD Values of valsartan and sacubitril

Parameter	Valsartan (µg)	Sacubitril (µg)
LOD	0.004	0.004

Table No: 7 LOQ Values of valsartan and sacubitril

Parameter	Valsartan (µg)	Sacubitril (µg)
LOQ	0.012	0.012

Robustness

Table No: 8 Results of robustness of Sacubitril and Valsartan

Parameter	RT		Theoretical plates		Asymmetry	
	SAC	VAL	SAC	VAL	SAC	VAL
Decreased flow rate (0.6ml/min)	2.635	2.229	4820	4191	1.08	1.09
Increased flow rate (1.0ml/min)	3.930	3.316	3620	3123	1.06	1.05
Wave Length 265nm	3.159	2.664	4098	3598	1.07	1.07
Wave Length 269nm	3.158	2.665	4125	3627	1.07	1.07

Where SAC: Sacubitril; VAL: Valsartan)

Result

The results of Robustness of the present method had shown that changes made in the flow and wavelength did not produce significant changes in analytical results which were presented in the above table. As the changes are not significant, we can say that the method is robust.

Ruggedness (Intermediate Precision)

Table No: 9 Results of Ruggedness of Sacubitril and Valsartan

	%Assay		%RSD	
	SAC	VAL	SAC	VAL
Analyst-1	99.8	99.8	0.21%	0.14%
Analyst-2	99.5	99.6		

The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

Table No: 10 System suitability data of Sacubitril and Valsartan

Parameter	Sacubitril	Valsartan	Acceptance Criteria
Retention time (min)	3.156	2.663	±1.0
Theoretical plates	4088	3598	>3000
Tailing factor	1.07	1.07	<1.50
% RSD	0.28	0.22	<2.00

Table No: 11 Standard Results of Sacubitril and Valsartan

S.No	Sample Name	RT (min)		Area		USP plate count		USP tailing	
		SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
1.	Injection1	3.159	2.664	2011734	1128557	4098	3598	1.07	1.07
2.	Injection 2	3.159	2.665	2014764	1130463	4125	3627	1.07	1.07
3.	Injection 3	3.156	2.662	2009811	1128528	4105	3557	1.07	1.07
4.	Injection 4	3.15	2.66	2004468	1126557	4023	3535	1.07	1.07
5.	Injection 5	3.154	2.663	2000740	1123889	4090	3597	1.07	1.07

Result

Results of system suitability study are summarized in the above table. Five consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

CONCLUSION

The present experimental investigation reported in this thesis is to improve a new analytical method development and validation as per the ICH guidelines which are recommended for the analytical method validation. New analytical method developments were carried out for the simultaneous estimation of active pharmaceutical ingredients and its combined marketed dosage form. Sacubitril and Valsartan was the selected combination, marketed in the brand name Entresto (97mg of sacubitril and 103mg of valsartan). The selected drug was used for the simultaneous analysis and validation by reverse phase liquid chromatography.

The simultaneous estimation of Valsartan and Sacubitril in drug product was done by liquid chromatography and the chromatographic separation was achieved on C18 column (X- Terra RP-18 150*4.6mm) column at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol (25:75%v/v). The flow rate was 0.8ml/ min. and ultra violet detector at 267nm. The average retention time for Valsartan and Sacubitril found to be 2.66 min and 3.154 min. The % purity of Sacubitril and Valsartan was found to be 99.8% and 99.3%respectively.

FUTURE SCOPE

A new method was developed for the quantification of SAC and VAL in combined tablet formulation. This method seems to obey the validation parameters and cost effective. This method can be routinely employed for the analysis of this combination. The reason behind selection of two different categories of antihypertensive drugs was also justified properly because of synergistic effect on lowering of blood pressure and reduction of side effects of one drug by another. Side effects of the individual drugs can be mitigated by using a complementary agent rather than increasing the dose of a single agent. Hence the suggested RP-HPLC method can be used for routine analysis of Sacubitril and valsartan in Bulk and Pharmaceutical dosage form.

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