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Method Development and Validation for Valsartan and Sacubitril by RP-HLPC

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***Abstract*: The estimation of Saccubitril and Valsartan was done by RP-HPLC. The assay of Saccubitril and Valsartan was performed with tablets and the % assay was found to be 99.11and 100.76. and it's linearity was found to be linear with a correlation coefficient of 0.999 and 0.99. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.43 and 0.68. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.61 and 0.85. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.34% and 100.22% for Saccubitril and Valsartan. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Saccubitril was found to be 3.02 and 9.98 and LOD and LOQ for Valsartan was found to be 3.00 and 10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit.**

***Keywords*: RP–HPLC, Saccubitril, Valsartan.**

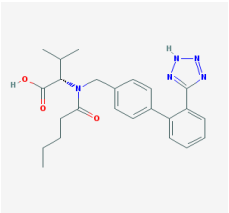
1. Introduction

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

2. Drug Profile

A. Valsartan

Structure:



IUPAC name: (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)pentanamido]butanoic acid

Molecular weight: 435.519 gm/mol

Molecular formula: $C_{24}H_{29}N_5O_3$.

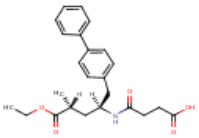
Solubility: soluble in pure methyl acetate, n-butyl acetate, acetonitrile, N, N-dimethyl formamide, dichloromethane, chloroform and ethanol.

Category: Anti-hypertensive drug, Angiotensin receptor blocker.

B. Sacubitril

Synonym: Sacubitril impurity 1

Structure:



IUPAC name:
3-{[(2S,4R)-1-{[1,1'-biphenyl]-4-yl}-5-ethoxy-4 methyl5,2oxopentanyl]carbamoyl}propanoic acid.

Molecular weight:

Average: 411.498 Monoisotopic :411.204573038

Molecular formula: $C_{24}H_{29}NO_5$

Solubility: Soluble in water, DMSO

Category: Anti-hypertensive drug

3. Experimental Method

Table 1
Instruments used

S. No.	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

A. HPLC method development

1) Wave length selection

UV spectrum of 10 µg/ml Sacubitril and Valsartan in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 267nm. At this wavelength both the drugs show good absorbance.

2) Optimization of Column

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Symmetry (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

B. Chemicals used

1) Optimized chromatographic conditions

Instrument used: Waters HPLC with auto sampler and uv or detector.

Temperature: Ambient
Column: Symmetry (4.6*250mm, 5microns)
Buffer: Phosphate buffer
pH: 3.5
Mobile phase: 30% buffer 70% Methanol
Flow rate: 1 ml per min
Wavelength: 267 nm
Injection volume: 20 µl
Run time: 6 min.

Table 2
Chemical used

1	Saccubitril	Supplied by Pharmatrain
2	Valsartan	Supplied by Pharmatrain
3	KH_2PO_4	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

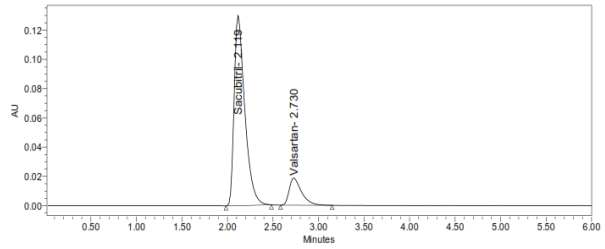


Fig. 1. Optimized Chromatogram for Saccubitril and Valsartan

C. Preparation of buffer and mobile phase

1) Preparation of Phosphate buffer

Accurately weighed 1.732g of potassium dihydroger ortho phosphate was taken in a 500ml volumetric flask, dissolved and diluted to 500ml with HPLC water and the volume was adjusted to pH 3.5 with OPA.

2) Preparation of mobile phase

Accurately measured 500 ml (30%) of above buffer and 500 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

3) Diluent Preparation

The Mobile phase was used as the diluent.

D. Preparation of the saccubitril and valsartan standard and sample solution

1) Standard Solution Preparation

Accurately weigh and transfer 12 & 13mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

2) Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 12 & 13mg of Sacubitril & Valsartan sample into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3) Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Saccubitril and Valsartan peaks and calculate the %Assay by using the formulae.

E. System suitability

Tailing factor for the peaks due to Saccubitril and Valsartan in Standard solution should not be more than 2.0

Theoretical plates for the Saccubitril and Valsartan peaks in Standard solution should not be less than 2000.

Resolution for the Saccubitrile and Valsartan peaks in standard solution should not be less than 2.

F. Linearity

1) Preparation of stock solution

Accurately weigh and transfer 12 & 13mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

From the above stock solution pipette out 1,2,3,4,5 ml of above stock solutions has taken in different 10ml of volumetric

flasks, dilute up to the mark with diluent.

2) Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

G. Precision

Preparation of solutions: Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

H. Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of solution: Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

I. Accuracy

Preparation of standard solution: Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 6 & 6.5mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 12 & 13mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 18 & 19.5mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Saccubitril & Valsartan and calculate the individual recovery and mean recovery values.

J. Detection limit

Limit of detection: (for Saccubitril)

Preparation of 36µg/ml solution:

Pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.02 µg/ml solution:

Further pipette 0.1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Further pipette 0.65ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

K. Limit of quantification

Preparation of 36 µg/ml solution:

Pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.06 µg/ml solution:

Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Further pipette 0.16 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Limit of detection: (for Valsartan)

Preparation of 39 µg/ml solution:

Accurately weigh and transfer 12 & 13mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.14 µg/ml solution:

Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Further pipette 0.38 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

L. Limit of quantification

Preparation of 39µg/ml solution:

Accurately weigh and transfer 12 & 13mg of Saccubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3.0ml of Saccubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.46 µg/ml solution:

Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Further pipette 1.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure for LOD and LOQ:

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

M. Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 36 ppm & 39 ppm of Saccubitril & Valsartan prepared and analysed using the varied flow rates along with method flow rate.

The results are summarized.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

The method is robust in less flow condition.

b) Standard solution 36 µg/ml & 39 µg/ml of Sacubitril & Valsartan was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized,

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

4. Results and Discussion

Specificity:

Auto-Scaled Chromatogram

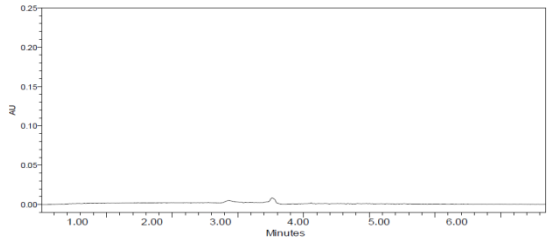


Fig. 2. Chromatogram of blank

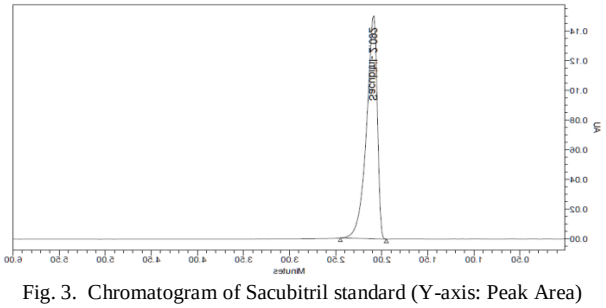


Fig. 3. Chromatogram of Sacubitril standard (Y-axis: Peak Area)

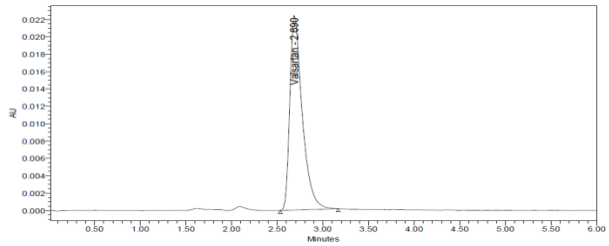


Fig. 4. Chromatogram of Valsartan standard

Table 2
System suitability parameters for Sacubitril and Valsartan

Peak Name	Retention time	Area	Height	Resolution	USP Plate count	USP Tailing
Sacubitril	2.119	1051860	130126		2524.84	1.6
Valsartan	2.730	173574	18560	3.1	3177.99	1.4

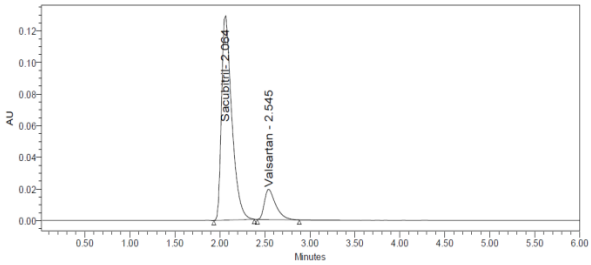


Fig. 5. Chromatogram of sample

System suitability studies:
Linearity:
Linearity of Sacubitril and Valsartan

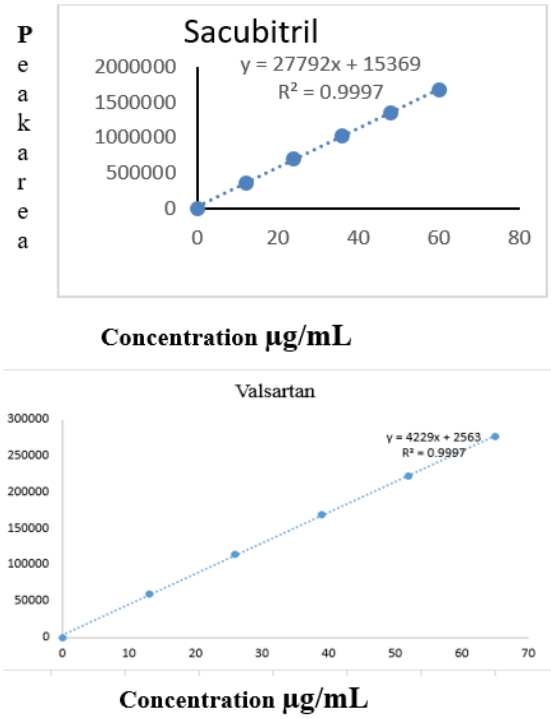


Fig. 6. Curve calibration for and Valsartan

Table 3

Table 4
Linearity results of Valsartan

S. No.	Linearity Level	Concentration(µg/ml)	Area
1	I	13	59045
2	II	26	114337
3	III	39	168147
4	IV	52	220495
5	V	65	276005
Correlation Coefficient			0.999

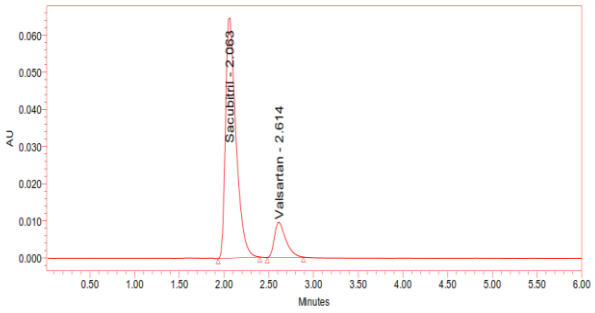


Fig. 7. Accuracy 50% results of Sacubitril and valsartan

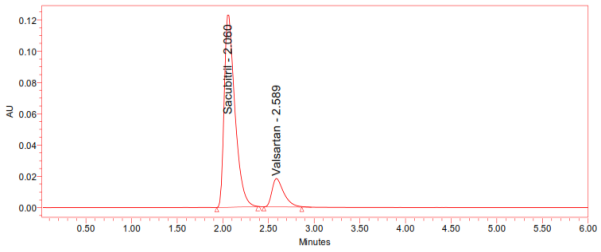


Fig. 8. Accuracy 100% results of Sacubitril and valsartan

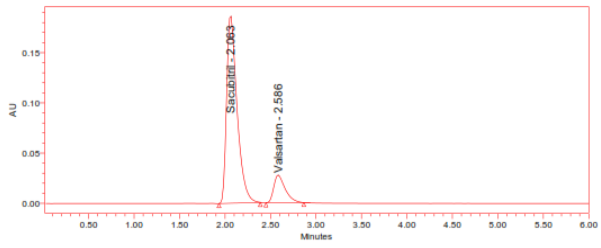


Fig. 9. Accuracy 150% results of Sacubitril and valsartan

Table 5
Accuracy results for saccubitril

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	526473	6	6	100.2%	100.34%
100%	1011675	12	12.10	100.85	
150%	1481480	18	18.03	100.15	

Table 6
Accuracy results of Valsartan

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	620862	6.5	6.48	99.62%	100.27%
100%	1194715	13	13.08	100.59%	
150%	1792072	19.5	19.62	100.61%	

Precision:

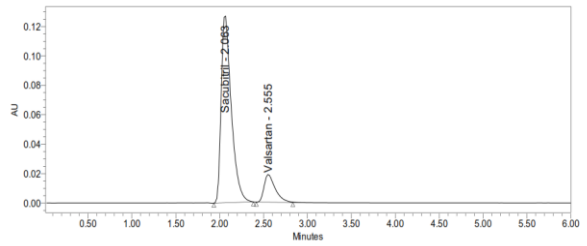


Fig. 10. Precision results for Saccubitril

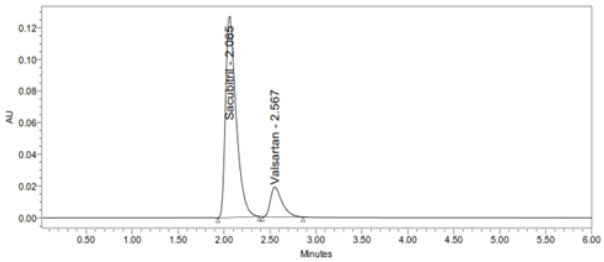


Fig. 11. Precision results for Valsartan

Table 7
Precision results for Saccubitril

Injection	Area
Injection-1	1023945
Injection-2	1027796
Injection-3	1026845
Injection-4	1036375
Injection-5	1020865
Average	1027165.2
Standard Deviation	5817.7
% RSD	0.8

Table 8
Precision results for Valsartan

Injection	Area
Injection-1	168040
Injection-2	167914
Injection-3	170372
Injection-4	175848
Injection-5	166068
Average	169648.2
Standard Deviation	3787.4
%RSD	0.5

Intermediate precision:

Table 9
ID Precision results are summarized Sacubitril

Injection	Area
Injection-1	1003903
Injection-2	1018214
Injection-3	1012117
Injection-4	1018518
Injection-5	1009168
Injection-6	1020368
Average	1013714.4
Standard Deviation	6435.6
% RSD	0.6

Table 10
ID Precision The results are summarized Valsartan

Injection	Area
Injection-1	164423
Injection-2	165485
Injection-3	166719
Injection-4	165469
Injection-5	166045
Injection-6	165226
Average	165561.0
Standard Deviation	774.2
% RSD	0.5

LOD:

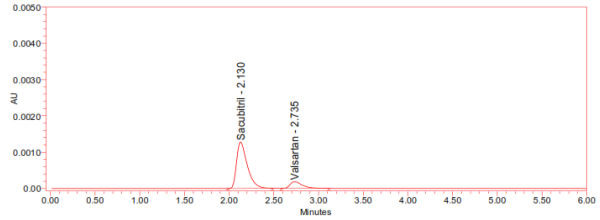


Fig. 12. LOD Chromatogram for Sacubitril and Valsartan

Sacubitril:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 66 µV
Signal Obtained from LOD solution: 198 µV
S/N = 1989/66 =3.00
Acceptance Criteria:
S/N Ratio value shall be 3 for LOD solution.
Valsartan:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 66 µV
Signal Obtained from LOD solution: 199 µV
S/N = 199/66 = 3.02
Acceptance Criteria:
S/N Ratio value shall be 3 for LOD solution.

LOQ:

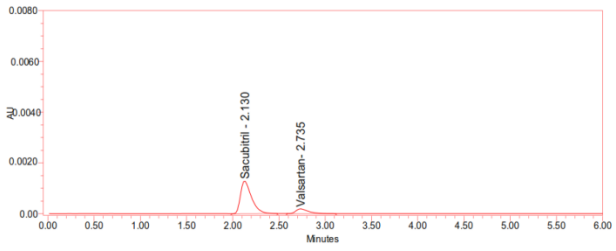


Fig. 13. LOQ Chromatogram for Sacubitril and Valsartan

Sacubitril:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank: 66 µV
Signal Obtained from LOQ solution: 659µV
S/N = 659/66 = 9.98
Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

Valsartan:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 66 µV

Signal Obtained from LOQ solution: 660µV

S/N = 660/66 = 10.00

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Robustness:

Less Flow:

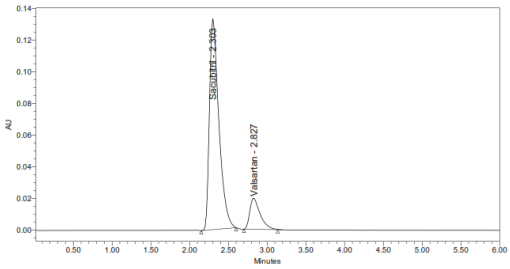


Fig. 14. Chromatogram for less flow

More flow:

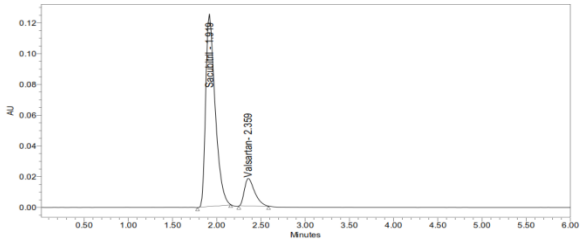


Fig. 15. Chromatogram for more flow

Robustness studies for Sacubitril and Valsartan:

Table 11
Robustness studies for Sacubitril

S. No.	Flow Rate (ml/min)	Robustness Results	
		USP Plate Count	USP Tailing
1	0.9	2680.7	1.3
2	1	2524.84	1.3
3	1.1	2124.4	1.3

Table 12
Robustness results for Valsartan

S. No.	Flow Rate (ml/min)	Robustness Results	
		USP Plate Count	USP Tailing
1	0.9	3200.8	1.3
2	1	3177.99	1.4
3	1.1	2973.7	1.4

Assay Method:

Standard preparations are made from the API and sample preparations are from Enteresto tablet. Both sample and standards are injected three homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.11% and 100.76% for Saccubitril and Valsartan respectively.

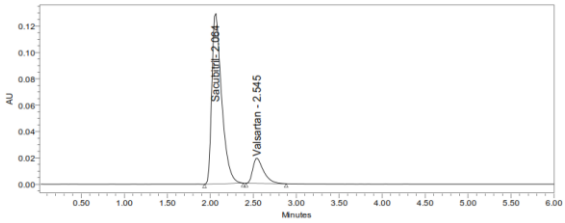


Fig. 16. Assay Chromatogram Sample

Assay Results: (Sacubitril)

1043363 12 1 3 10 10 10 261
----------x--------x-------x-------x--------x--------x-------x-----
1050653 10 10 10 130.5 1 3 24

99.8
-------x100= 99.11%
100

Assay Results: (Valsartan)

174732 13 1 3 10 10 10 261 99.8
----------x-------x------x-------x--------x------x------x--------x--
173068 10 10 10 130.5 1 3 26 100

x100= 100.76%

5. Summary and Conclusion

The estimation of Saccubitril and Valsartan was done by RP-HPLC. The assay of Saccubitril and Valsartan was performed with tablets and the % assay was found to be 99.11and 100.76 which shows that the method is useful for routine analysis. The linearity of Saccubitril and Valsartan was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.43 and 0.68 for Saccubitril and Valsartan which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.61 and 0.85 for Saccubitril and Valsartan which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.34% and 100.22% for Saccubitril and Valsartan. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Saccubitril was found to be 3.02 and 9.98 and LOD and LOQ for Valsartan was found to be 3.00 and 10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision

under given set of conditions.

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