Uppalapati.Jyothi et al /J. Pharm. Sci. & Res. Vol. 10(9), 2018, 2201-2204

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Sacubitril and Valsartan in Drug Product

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Abstract:

The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Valsartan and Sacubitril in drug product by liquid chromatography. The chromatographic separation was achieved on C8 column (Luna C8 150*4.6, 3um) at ambient temperature .The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Methanol (25:75). The flow rate was 1.0ml/ minute and ultra violet detector at 267nm. The average retention time for Valsartan and Sacubitril found to be 3.049 min and 3.991 min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 51.5 - 154.5µg/mL for Valsartan and 48.5 -145.5µg/mL of Sacubitril.

Key words: Valsartan, Sacubitril, Isocratic, HPLC, Luna, Trifluoro acetic acid, Acetonitrile, Methanol and validation

1. INTRODUCTION

Valsartan

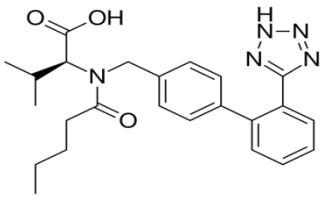


Fig. 1. Chemical structure: Valsartan

Valsartan is an angiotensin II receptor commonly called angiotensin receptor blocker, Valsartan is mainly used for treatment of high blood pressure, congestive heart failure, and to increase the chances of living longer after a heart attack.

Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure. The drug binds to angiotensin type I receptors (AT1), working as an antagonist. This mechanism of action is different than the ACE inhibitor drugs, which block the conversion of angiotensin I to angiotensin II.

Valsartan is chemically designated as (S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido)butanoic acid. Its molecular formula is C24H29N5O3, and its molecular weight is 435.519 g/mol.

Sacubitril

Sacubitril is an antihypertensive drug , Sacubitril is a prodrug that is activated to Sacubitrilat by de-ethylation via esterase, Sacubitril inhibits the enzyme neprilysin, which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume. In addition, neprilysin degrades a variety of peptides including bradykinin, an inflammatory mediator exerting potent vasodilatory action.

Sacubitril is chemically designated as 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid. Its molecular formula is C24H29NO5, and its molecular weight is 411.49 g/mol.

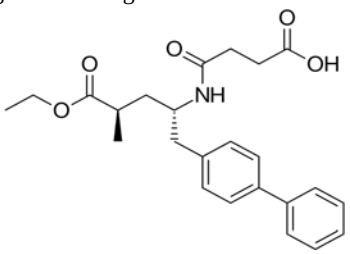


Fig. 2. Chemical structure: Sacubitril

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C8 column (Luna C8 150*4.6,3µm) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance and Vaccum micro filtration unit with 0.45µ membrane filter.

2.2 Materials: Pharmaceutically pure sample of Valsartan/Sacubitril were obtained as gift samples from Fortune pharma training institute, Sri Sai nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents pvt ltd. Triflouro acetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on a (Luna C8 150*4.6, 3 µm) C8 column, aided by mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water : Methanol (25:75). The flow rate was 1.0 ml/ minute and ultra violet detector at 267nm, that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation: Taken accurately 1ml of Trifluoro acetic acid in 1000mL of water

Mobile phase: Then added 25 volumes of buffer and 75 volumes of Methanol mixed well and sonicated for 5 min.

Diluent: Water: Acetonitrile: 50:50 v\v

2.4 Preparation of solutions

2.4.1 Standard solution: 51.5 mg of pure Valsartan and 48.5 mg 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 103µg/ml of Valsartan and 97 µg/ml Sacubitril .

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 103mg of Valsartan and 97mg of Sacubitril sample and transferred to 100 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 1 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 103 µg/ml of Valsartan and 97 µg/ml Sacubitril .

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 51.5 -154.5µg/ml for Valsartan and and 48.5 -145.5µg/ml 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 145.5 µg/mL of Sacubitril and 51.5g/mL,77.25µg/mL ,103µg/mL ,128.75µg/mL and 154.5 µg/mL Valsartan.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

$LOD = 3.3\ \delta/S$

$LOQ = 10\ \delta/S$

Where,

δ = 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.

2.5.4. Method precision

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

2.5.5. 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.

2.5.6. 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.

2.5.7 Forced degradation studies

The study was intended to ensure the effective separation of Sacubitril, Valsartan and its degradation peaks of formulation ingredients at the retention time of Sacubitril and Valsartan. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods.

Acid degradation Forced degradation in acidic media was performed by keeping the standard solution in contact with 0.1 N HCl for 3h at room temperature. After 3h the solution was neutralized with 0.1 N NaOH and solution was diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 97 µg/ml of Sacubitril and 103 µg/ml of Valsartan.

Alkaline degradation Forced degradation in basic media was performed by keeping the standard solution in contact with 0.1 N NaOH for 3h at room temperature. After 3h the solution was neutralized with 0.1 N HCl and solution was diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 97 µg/ml of Sacubitril and 103 µg/ml of Valsartan.

Oxidation degradation Forced degradation in 5% H2O2 media was performed by keeping the standard solution in contact with 5% H2O2 for 3h at room temperature. After 3h, solution was diluted with mobile phase up to 10 ml to achieve the appropriate concentration 97 µg/ml of Sacubitril and 103 µg/ml of Valsartan.

Thermal degradation Sample solution was exposed to temperature of 105°c for 24h in an oven. After 24h, solution was diluted with mobile phase up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration 97 µg/ml of Sacubitril and 103 µg/ml of Valsartan.

Photolytic degradation Sample solution was exposed in the sunlight for 24h. After 24h. Solution was diluted with mobile phase up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration 97 µg/ml of Sacubitril and 103 µg/ml of Valsartan.

3. Results And Discussions:

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 upto the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 267nm

After several initial trails with mixtures of methanol, water, Acetinitrile 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). at flow rate was 1.0 mL/ minute brought sharp peaks. The chromatogram was shown in Fig 3**.**

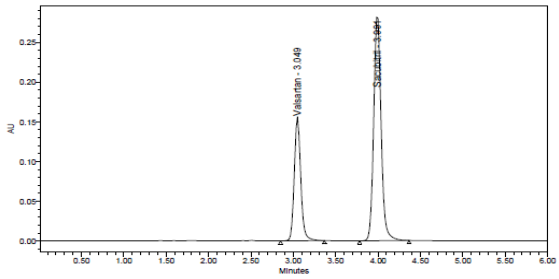


Fig 3 Chromatogram of Valsartan and Sacubitril

System suitability

The system suitability of the method was checked by repeated preparations for Sacubitril and Valsartan. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system, System suitability data of Sacubitril and Valsartan are shown in Table 1

Parameter	Valsartan	Sacubitril	Acceptance criteria
Retention time	3.051	3.996	+-10
Theoretical plates	7669	10547	>3000
Tailing factor	1.13	1.12	<1.50
% RSD	0.20	0.15	<2.00

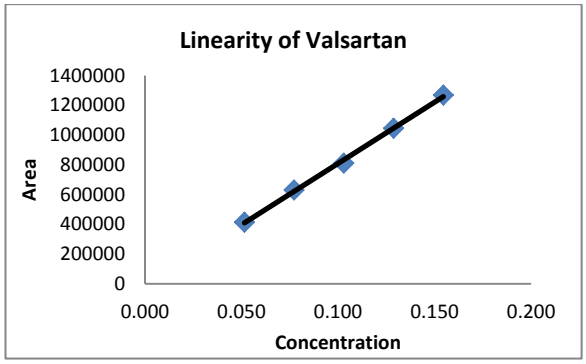
Table 1 System suitability data of Valsartan and Sacubitril

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 51.5 -154.5µg/ml for Valsartan and and 48.5 -145.5µg/ml 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 145.5 µg/mL of Sacubitril and 51.5g/mL,77.25µg/mL ,103µg/mL ,128.75µg/mL and 154.5 µg/mL Valsartan. The linearity data for Valsartan and Sacubitril are shown in Table 2 and Table 3

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 0.9992 and 0.9993 for Valsartan and Sacubitril.



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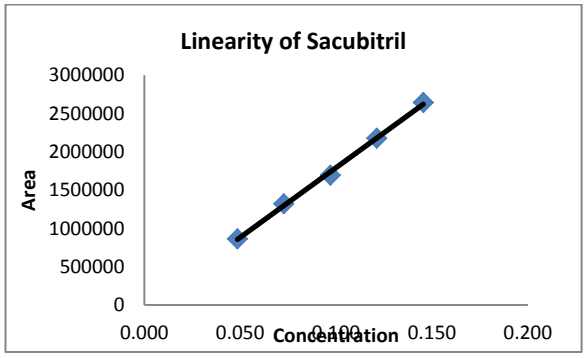

B

Fig. 4 Calibration curve: (A) Valsartan: (B) Sacubitril

Level	Concentration (mg/mL)	Peak area
50%	0.052	413825
75%	0.077	360320
100%	0.103	811399
125%	0.129	1045951
150%	0.155	1268092
Correlation		**0.9992**

Table 2 : Linearity data of Valsartan

Level	Concentration (mg/mL)	Peak area
50%	0.049	861882
75%	0.073	1318917
100%	0.097	1694614
125%	0.121	2175578
150%	0.146	2641781
Correlation		**0.9993**

Table 3: Linearity data of Sacubitril

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$LOD = 3.3\ \sigma / S \quad \ldots\ldots\ldots (1)$$

$$LOQ = 10\ \sigma / S \quad \ldots\ldots\ldots (2)$$

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

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

	Valsartan mg	Sacubitril mg
LOD	0.006	0.006
LOQ	0.018	0.017

Table 4 LOD and LOQ values Calculated from calibration curve

Method precision (repeatability)

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 measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Valsartan and Sacubitril are shown in Table 5 and Table 6.

	Retention time	Peak area	% Assay
1	3.052	824318	99.1
2	3.054	828866	99.8
3	3.048	822025	98.4
4	3.056	815480	99.6
5	3.056	831875	98.8
6	3.047	830594	99.6
Mean	3.052	825526	99.2
%RSD	**0.13**	**0.75**	**0.56**

Table 5: Summary of peak areas for method precision of Valsartan

Sample No	Retention time	Peak area	% Assay
1	4.004	1721132	99.4
2	4.000	1730572	100.2
3	3.987	1715916	98.9
4	4.012	1702668	98.2
5	4.003	1737846	99.8
6	3.988	1732424	100.1
Mean	3.999	1723426	99.4
%RSD	**0.24**	**0.75**	**0.77**

Table 6: Summary of peak areas for method precision of Sacubitril

Accuracy (recovery study):

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. The percentage recovery results obtained are listed in Table 7 &8

LEVEL	S.No	%Recovery of Valsartan	Average
50	1	99.2	99.3%
	2	100.0	
	3	98.6	
100	1	99.1	99.1%
	2	99.8	
	3	98.4	
150	1	99.9	99.7%
	2	99.5	
	3	99.6	

Table 7: Recovery data of Valsartan

LEVEL	S.No	%Recovery of Sacubitril	Average
50	1	99.4	99.9%
	2	99.8	
	3	100.5	
100	1	99.4	99.5%
	2	100.2	
	3	98.9	
150	1	98.6	99.3%
	2	99.6	
	3	99.7	

Table 8: Recovery data of Sacubitril

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. The results were shown in (Table 9&10) The results of Robustness of the present method had shown that changes are not significant was found to be the method is Robust.

parameter	Rt of Valsartan	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	3.087	8503	1.17
Increased flow rate (1.2ml/min)	2.081	6376	1.15
Wave Length 265nm	3.049	7584	1.13
269	3.048	7714	1.12

Table 9: Results of of Valsartan

parameter	Rt of Sacubitril	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	3.728	10826	1.17
Increased flow rate (1.2ml/min)	2.506	7642	1.13
Wave Length 265nm	3.991	10671	1.13
269	3.991	10622	1.13

Table 10: Results of of Sacubitril

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table 11&12.
The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

		%Assay	%RSD
Analyst-1	VALSARTAN	99.1	0.50%
Analyst-2		99.8	

Table 11: Ruggedness data for Valsartan

		%Assay	%RSD
Analyst-1	SACUBITRIL	99.4	0.57%
Analyst-2		100.2	

Table 12: Ruggedness data for Sacubitril

S.No	Degradation Condition	Valsartan		Sacubitril	
		Degradation (%)	Active drug present after degradation (%)	Degradation (%)	Active drug present after degradation (%)
1	Acid degradation 0.1N HCl 3h	2.29	97.71	25.70	74.30
2	Base degradation 0.1N NaOH 3h	5.31	94.69	20.48	79.52
3	Peroxide degradation 5% H_2O_2 3h	1.81	98.19	20.12	79.88
4	Thermal degradation 24 h	328	96.72	0.30	99.70
5	Photolytic degradation 24h	1.34	98.66	0.67	99.33

Table 13: Summary of Degradation data for Valsartan and Sacubitril

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of VALSARTAN and SACUBITRIL 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 pharmaceutical industries, approved testing laboratories.

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