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Development and Validation of A RP- HPLC Method for the Simultaneous Estimation of Valsartan and Sacubitril in Rat Plasma

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Abstract

A basic precise selective, sensitive isocratic technique was developed and valid for the quantitative synchronic estimation of Sacubitril and Valsartan drug in rat plasma was by RP- HPLC system. The chromatographic activity separation was administrated out on Intersil C18 (250 x 4.6mm, 5µm) column with a mix of acetonitrile: di-potassium hydrogen phosphate, pH 3.0 adjusted with Phosphate buffer (30:70%v/v) as mobile part. The analytes were eluted with a rate of flow of 0.8ml/min and at a wavelength of 371nm of UV detection. The strategy was valid for precision, accuracy, linearity, Limit of detection, Limit of Quantification, Ruggedness following the ICH guidelines.

The retention time was 10.725min and 15.366min and the system suitableness results was 99.95% and 100.24% for sacubitril and valsartan respectively. Linearity contemplate was completed between 100-500µg/ml and 5µg-25µg/ml, linear regression coefficient was observed to be 0.999 and the percentage recovery varies from 98-102% of Sacubitril and valsartan. No interference from any part of bulk and pharmaceutical dosage form was determined. All the parameters of validation are found to be at intervals within the vary that confirms the quality of the strategy for the determination of Sacubitril and Valsartan.

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

Introduction

High Performance Liquid Chromatography

Pittcon paper, originally indicated the proven fact that prime air mass was wont to generate the flow required for liquid natural action in packed columns. Among the beginning, pumps only had a pressure capability of 500 psi. This was known as air mass liquid natural action, or HPLC. New HPLC instruments could develop up to 6,000 psi of pressure, and incorporated improved injectors, detectors, and columns [1-6]. With continued advances in performance throughout this time (smaller particles, even higher pressure), the descriptor HPLC remained an analogous, but name was changed to high performance liquid natural action.

Reversed Phase Chromatography

Reversed phase mode is the most prevalent mode for scientific and preparative partitions of mixes of worry in biological products, pharmaceutical plans and API's, substance substances, nourishment and biomedical designing. The stationary stage is non-polar hydrophobic pressing with octyl and octadecyl useful gathering attached to silica gel and the mobile stage is a polar dissolvable, regularly a mostly or completely watery versatile stage [7-11]. Polar substances lean toward the versatile stage and elute first. Maintenance increments as the hydrophobic character of the solutes expands, by and large, the lower the extremity of the versatile stage, higher is the eluent quality.

Drug Profile

Sacubitril is chemically 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl] amino}-4-oxobutanoic acid which is an antihypertensive drug used in combination with valsartan for the treatment of heart failure. Sacubitril could be a prodrug that's activated to sacubitril at (LBQ657) by de-ethylation via esterases. Sacubitril at inhibits the catalyst neprilysin, that is accountable for the degradation of chamber and brain symptom organic compound, a pair of blood pressure-lowering peptides that employment within the main by reducing blood volume. Valsartan is chemically (2S)-3-methyl-2-[pentanoyl- [[4- [2-(2H-tetrazol-5- yl) phenyl] phenyl] methyl] amino] butanoic acid. Valsartan is Associate in Nursing man of affairs that by selection inhibits the binding of Hypertensin to AT1, that's is found in several tissues like tube-shaped structure sleek muscle and in addition the adrenal glands [8-12].

This effectively inhibits the AT1-mediated agent vasoconstrictive and aldosterone-secreting effects of Hypertensin and finally ends up during a decrease in tube-

shaped structure resistance, and force per unit area. Valsartan is selective for AT1 and has regarding affinity for AT2. Inhibition of mineralocorticoid secretion might inhibit metallic element and water organic process among the kidneys whereas decreasing excretion. The primary matter of valsartan, valeryl 4-hydroxy valsartan, has no medicine activity. Literature search reveals that only two analytical methods were reported for simultaneous estimation of sacubitril and valsartan from rat plasma using LC-MS/MS and from a synthetic mixture using HPLC [12-20]. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for the simultaneous estimation of sacubitril and Valsartan from rat plasma (Figures 1 & 2).

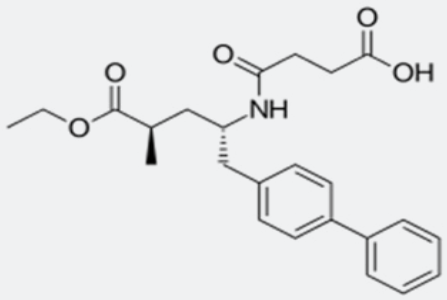

Figure 1: Peripheral Mechanism of Appetite Regulation.

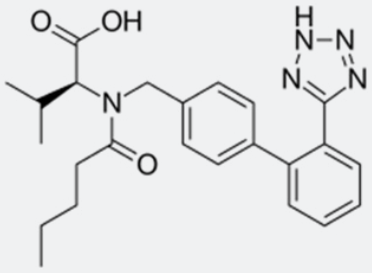

Figure 2: Chemical structure of Valsartan.

Material and Methods

Materials and Reagents

Sacubitril and Valsartan, KH2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, di-potassium hydrogen phosphate, Ortho phosphoric Acid.

Equipment

HPLC-WATERS, software: Empower, 2695 separation module, PDA detector, UV/VIS spectrophotometer LABINDIA UV 3000+, pH meter, Weighing machine.

Preparation of The Sacubitril & Valsartan Standard & Sample Solution

Standard Solution Preparation

10 mg Amount of standard was mixed with 10 ml acetonitrile to +2ml of rat plasma (untreated) then vertically shaked for 30 min then centrifuged at 5000rpm for 1 hr. Then it was filterated using membrane filters to get clear organic solution. Then it was filled in to the sample vials of HPLC and loaded on to HPLC for Run.

Sample Solution Preparation

Blood samples are collected from the animal's rats and then centrifuged at 5000rpm for 1 hr to separate the plasma from blood. Then the separated was mixed with acetonitrile then loaded on to the HPLC for Run.

Results and Disscusion

Method Development

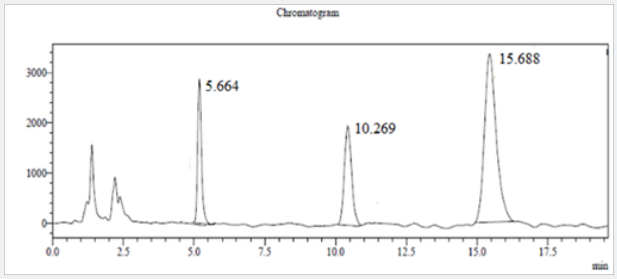

Figure 3: Optimsed Chromatogram of Sacubitril and Valsartan.

Chromatographic Conditions (OptimisedMethod) (Figure 3)

Mobile phase : Phosphate buffer pH 3.0: Methanol (30:70%v/v)

Column : Inertsil C18 5µm (4.6x250mm)

Flow rate : 0.8 ml/min

Wavelength : 371 nm

Column temp : Ambient

Sample Temp : Ambient

Injection Volume: 10 µl

Table 1: Details of Optimised Method.

S. No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Sacubitril	10.269	123649	642415	5214	1.3	7.1
2	Valsartan	15.688	564121	393414	8745	1.2	

(Table 1) The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as optimized method. The developed Method was validated for linearity, precision, accuracy, robustness and is applied for forced degradation studies as per the ICH guidelines.

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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 (Table 2).

Table 2: Results of system suitability parameters for Sacubitril and Valsartan.

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Sacubitril	10.221	124505	213642		1.2	4673.4
2	Valsartan	15.411	1308495	154566	6 0	1.3	6090.3

Linearity

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Sacubitril and Valsartan (marketed formulation) sample into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Tables 3 & 4) (Figures 4 & 5).

Table 3: Linearity of Valsartan and Sacubitril.

Sample ID	Valsartan		Sacubitril	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating concentration	20	329217	20	136108
40% of operating concentration	40	628085	40	261946
60% of operating concentration	60	908859	60	372984
80% of operating concentration	80	1196986	80	487383
100% of operating concentration	100	1536686	100	617463
Correlation Coefficient			0.999	

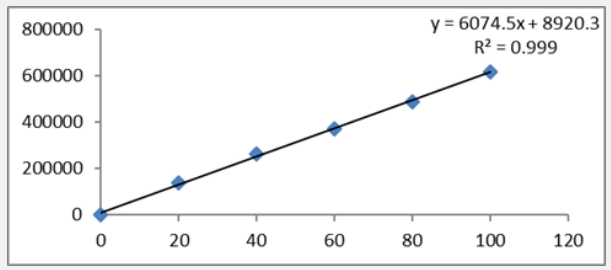

Figure 4: Chemical structure of Valsartan.

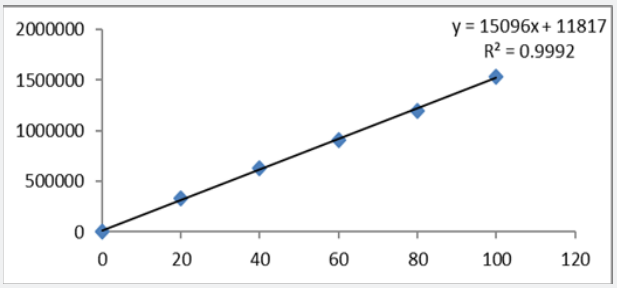

Figure 5: Calibration graph for Sacubitril.

Table 4: Analytical performance parameters of Sacubitril and Valsartan.

Parameters	Sacubitril	Valsartan
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R2)	0.999	0.999

Accuracy

Accurately weigh and transfer 10 mg of Sacubitril and Valsartan10mg of working standard into a 10ml& 100ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Tables 5 & 6).

Table 5: Accuracy (recovery) data for Sacubitril.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656459.5	5.0	5.036	100.7%	99.84%
100%	1308258	10.0	10.003	100.0%	
150%	1854208	14.4	14.224	98.780%	

Table 6: Accuracy (recovery) data for Valsartan.

% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
(at specification Level)	Area	Amount Added	5.036	100.7%	99.84%
(mg)	Amount Found	10.0	10.003	100.0%	
(mg)	% Recovery	Mean Recovery	14.224	98.780%	
50%	65800	5.3	5.33	100.8%	100.51%

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100%	124453	10	10.10	100.01%	
150%	187940	14.3	14.42	99.68%	

Precision

Table 7: Analytical performance parameters of Sacubitril and Valsartan.

Injection	Area
Injection-1	1304729
Injection-2	1303947
Injection-3	1302236
Injection-4	1304977
Injection-5	1308759
Average	1305529.8
Standard Deviation	2951.1
%RSD	0.2

Accurately weigh and transfer 25 mg of Sacubitril and Valsartan working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Tables 7 & 8).

Table 8: Analytical performance parameters of Sacubitril and Valsartan.

Injection	Area
Injection-1	123249
Injection-2	121766
Injection-3	124371
Injection-4	124591
Injection-5	124856
Average	124762.7
Standard Deviation	735.6
%RSD	0.6

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method,

Precision was performed on different day by using different make column of same dimensions (Tables 9 & 10).

Table 9: Results of Intermediate precision for Sacubitril.

Injection	Area
Injection-1	1300149
Injection-2	1342520
Injection-3	1365937
Injection-4	1306876
Injection-5	1308719
Average	1305870.2
Standard Deviation	3051.8
%RSD	0.2

Table 10: Results of Intermediate precision for Valsartan.

Injection	Area
Injection-1	122487
Injection-2	122826
Injection-3	122432
Injection-4	122732
Injection-5	122969
Average	122881.8
Standard Deviation	173.8
%RSD	0.1

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 (Tables 11-14).

Table 11: Flow Rate (ml/min) data for Sacubitril.

S. No		System Suitability Results	
	Flown Rate (ml/min)	USP Plate Count	USP Tailing
1	0.6	5439.9	1.3
2	0.8	4573.4	1.4
3	1	5226	1.4

Table 12: Flow rate (ml/min) data for Valsartan.

S. No		System Suitability Results	
	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.8	7053.3	1.3
2	1	6080.3	1.3
3	1.2	6988	1.2

Table 13: Change in Organic Composition in the Mobile Phase for Sacubitril.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.4
2	*Actual	4573.4	1.3
3	10% more	4618.1	1.4

Table 14: Change in Organic Composition in the Mobile Phase for Valsartan.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6587.7	1.2
2	*Actual	6080.3	1.3
3	10% more	6292.5	1.2

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Limit of Detection

Limit of detection: (For Sacubitril)

Accurately weigh and transfer 10 mg of Sacubitril working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection (For Valsartan)

Accurately weigh and transfer 10mg of Valsartan working standard into a 100ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Table 15).

Table 15: Results of LOD.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Sacubitril	52	152	2.9
Valsartan	52	156	3

Limit of Quantification

Limit of Quantification (for Sacubitril)

Accurately weigh and transfer 10 mg of Sacubitril working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification (for Valsartan)

Accurately weigh and transfer 10mg of Valsartan working standard into a 100ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Table 16).

Table 16: Results of LOQ.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Sacubitril	52	522	10.03
Valsartan	52	524	10.1

Summary and Conclusion

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of valsartan and sacubitril in bulk and tablet dosage form. Thus the validated economical method was applied for forced degradation study of valsartan and sacubitril tablet. High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Sacubitril and Valsartan was done by RP-HPLC in rat plasma. The Phosphate buffer was pH 3.0 and the mobile phase was optimized with consists of ACE: di-potassium hydrogen phosphate mixed in the ratio of 70:30 % v/ v.

Inertsil C18 column C18 (4.6 x 150mm, 5 µ m) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 371 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Sacubitril and Valsartan were found to be from 100-500 µ g/ml of Sacubitril and 1-5 µ g/ml of Valsartan. Linear regression coefficient was not more than 0. 999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Sacubitril and valsartan. LOD and LOQ were found to be within limit.

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