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A Validated RP-HPLC Method Development and Validation for Sacubitril and Valsartan in Combine Pharmaceutical Dosage Forms

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A B S T R A C T

The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation of Valsartan and Sacubitril in pure drug form. Chromatographic separation was done using Terrosil C_{18} column having dimension of (100 mm x 4.6 mm) having particle size of 5.0 µm, with mobile phase consisting of Phosphate buffer (KH_2PO_4 and K_2HPO_4) pH 3 ±0.02 pH adjusted with ortho phosphoric acid and Acetonitrile (25:75 %v/v), flow rate was adjusted to 0.8 ml/min and detection wavelength at 254nm. The retention times of Valsartan and Sacubitril was found to be 2.589 and 3.711mins. The proposed method has been validated for accuracy, precision, linearity, robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Valsartan and Sacubitril was found in range of 0.2µg-0.6µg and 0.1µg-0.3µg and correlation coefficient was found to be 0.999 and 0.999% RSD for intermediate precision was found to be 0.1 and 0.2, for repeatability was 0.2 and 0.5, % mean recovery for Valsartan and Sacubitril was found to be 99.77% to 100.12% respectively. The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of Valsartan and Sacubitril in API and Pharmaceutical dosage forms.

Keywords: Valsartan, Sacubitril, RP-HPLC, Method development, Validation**.**

A R T I C L E I N F O

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1. Introduction

Valsartan, (S)-3-methyl-2-(N-{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4-yl] methyl} pentanamido) butanoic acid, Valsartan (trade name Diovan) is an angiotensin II receptor antagonist (commonly called an ARB, or angiotensin receptor blocker), that is selective for the type I (AT1) angiotensin receptor. 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 [1].

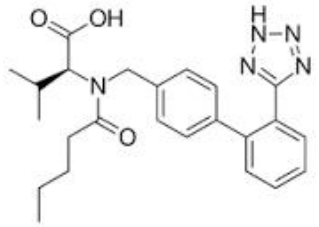

Figure 1: Structure of valsartan

Sacubitril, 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl -5-oxo-2-pentanyl]amino}-4-oxobutanoic acid, Sacubitril is a prodrug neprilysin inhibitor used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV) and reduced ejection fraction. It was approved under the FDA's priority review process for use in heart failure on July 7, 2015. Sacubitril's active metabolite, [3] LBQ657 inhibits neprilysin, a neutral endopeptidase that would typically cleave natiuretic peptides such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), c-type natriuretic peptide (CNP) [4, 5]. ANP and BNP are released under atrial and ventricle stress, which activate downstream receptors leading to vasodilation, natriuresis & dieresis [6].

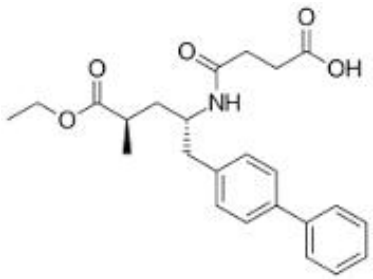

Figure 2: Structure of Sacubitril

Due to the lack of reported HPLC methods describing determination of the mixtures under investigation, it was deemed useful to develop simple, sensitive and selective HPLC method that could be useful for the simultaneous determination of Valsartan and Sacubitril. The proposed method was designed to be suitable for the quality assessment of these mixtures in a tablet dosage form.

2. Materials and Methods

Table 1: Active pharmaceutical Ingredient (pure drug)

S.No	Name	Specification
1	Valsartan and Sacubitril	Reference Standard

Table 2: Marketed Formulation

S.No	Name	Manufacturer
1	Valsartan	KP LABS
2	Sacubitril	KP LABS

Table 3: Chemicals used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC
2.	Methanol	Merck	HPLC
3.	Acetonitrile	Merck	HPLC
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R

Table 4: Solubility profile

Solvents	Valsartan	Sacubitril
Methanol	Insoluble	Insoluble
Acetonitrile	soluble	soluble
Water	Insoluble	Insoluble

Instrumentation:

The HPLC system was an LC Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 controller), in line degasser(Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Water's C-18 analytical column (150 mm × 4.6 mm inner diameter, 5 µm particle size, Waters, Dublin, Ireland) maintained at 45°C. The mobile phase was pumped at a flow rate of 1 mL min^{-1}.

Method Development [7,8,9]

Selection of mobile phase:

The method development and validation of Valsartan and Sacubitril requires greater resolution. Hence different solvent systems were tried.The trails are using UV 3000+ equipment with PDA detector and isocratic pump. The system controlled by LC solution software.

Selection of flow rate: The flow rate of Valsartan and Sacubitrilwere tried from 0.8 ml to 1.5ml.

Trial-1

Buffer preparation:

About 7.0g of potassium di hydrogen ortho phosphate was dissolved in 1000ml of HPLC grade water and P^H 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase:

Mobile phase consist of water : methanol HPLC of P^H 2.5 (30:70)was taken sonicated and degassed for 10 min and filtered through 0.45 µm nylon membrane filter.

Standard Preparation:

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it

was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution:

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Chromatographic conditions:

Column : Kromosil Column C_{18}(150mm x 4.6mm)5µg.
Mobile phase : Water: Methanol P^H 2.5 (30:70v/v)
Flow rate: 0.8ml/ min
Detector wavelength: 254 nm
Injection mode: Auto injector (vial)
Injection volume : 20µl

Preparation of mobile phase:

Mobile phase consist of buffer: Methanol of P^H2.5 (35:65) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter.

Standard Preparation:

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution:

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Chromatographic conditions:

Column : Thermosil C_{18}Column (100mm x 4.6mm) 5µg.
Mobile phase : Phosphate buffer: MethanolP^H2.5 (35:65v/v)
Flow rate : 1ml/ min
Detector wavelength : 254 nm
Injection mode : Auto injector (vial)
Injection volume : 20µl

Method Validation

The chromatographic conditions were validated by evaluating linearity, accuracy, method precision, limit of detection (LOD), limit of quantization (LOQ), ruggedness and robustness in accordance with ICH guidelines.

Specificity

Preparation of solutions

a) Placebo interference

Amount of 694.2mg of the capsule powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter.

Stock solution:

Further pipette 0.25ml of Valsartan and Sacubitril of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance criteria: Chromatogram of placebo should not show any peak at the retention time of analyte peak

Standard preparation

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Amount of 694.2mg of the tablet powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter.

Stock solution:

Further pipette 0.25ml of Valsartan and Sacubitril of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Linearity and Range

Preparation of stock solution

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The solution was mixed well and used for chromatographic injection.

Valsartan:

Preparation of linearity solution (20%)

0.2mlof stock solution was taken in 10mlof volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (30%)

0.3ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection

Preparation of linearity solution (40%)

0.4ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (50%)

0.5ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (60%)

0.6ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent The solution mixed well and used for chromatographic injection.

Sacubitril:

Preparation of linearity solution (10%):

0.1mlof stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (15%)

0.15mlof stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection

Preparation of linearity solution (20%)

0.2ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (25%)

0.25ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (30%)

0.3mlof stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluents. The solution mixed well and used for chromatographic injection.

Procedure: Each level of the above solutions was injected into the chromatographic system for five replicate and the peak area was measured. A graph was plotted (peak area versus concentration) and the correlation coefficient (r^2) was calculated.

Accuracy

Accuracy is the measure of exactness of an analytical method, or closeness of agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value. Accuracy is measured as the percentage of analyte recovered by assay, spiking samples in a blind study.

Preparation of stock solution

Weigh accurately 10mg Valsartan Working Reference Standard and15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions

50% Sample preparation

Weigh accurately 5.05mg Valsartan Working Reference Standard and 8.1mg of SacubitrilWorking Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

100% Sample preparation

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of SacubitrilWorking Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

150% Sample preparation

Weigh accurately 15mg Valsartan Working Reference Standard and 23.3mg of SacubitrilWorking Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

Procedure

The standard solution was injected in triplicate for Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

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

$$\textbf{\%Recovery} = \frac{\text{Sample peak area x weight of standard}}{\text{Standard peak area x weight of sample}} \text{ X } 100$$

Precision

Precision was the measure of the degree of repeatability of an analytical method under normal operation and it was normally expressed as the relative standard deviation for a statistically number of samples. Precision should be performed at three different levels: repeatability, intermediate precision and reproducibility.

Standard preparation

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of SacubitrilWorking Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Amount of 694.2mg of the tablet powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter.

(Stock solution)

Further pipette 0.25ml of Valsartan and Sacubitril of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Six replicate Sample solutions were prepared as per the test method and injected as per the test procedure

Intermediate Precision (Ruggedness)

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations

in mobile phase, different days etc. (i.e. from laboratory to laboratory, from analyst to analyst). Acceptance criteria for ruggedness, the % RSD for the area of five standard injections should not be more than 2%.

Standard preparation:

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution:

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Amount of 694.2mg of the tablet powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter.

Stock solution:

Further pipette 0.25ml of Valsartan and Sacubitril of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Five replicate Sample solutions were prepared as per the test method and injected as per the test procedure.

Robustness

As part of the robustness, deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method.

The flow rate was varied at 0.8ml/min to 1.2ml/min. Standard solution 10ppm of Valsartan and 15ppm of Sacubitril was prepared and analysed using the varied flow rates along with method flow rate.

The organic composition in the mobile phase was varied from 65% to75 % standard solution 10µg/ml of Valsartan and 15µg/ml of Sacubitril were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

$$LOD = 3.3 \times \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope

Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

System Suitability:

System is suitable for analysis if the relative standard deviation (RSD) of area counts in the six replicate injections for each peak should not be more than 2.0%. The USP plate count of peak should not be less than 2000 theoretical plates for HPLC. The tailing factor for each peak should not be more than 2.0 and the resolution for two peaks should not be less than 2.0. The results obtained indicate the good precision of the developed method.

3. Results and Discussion

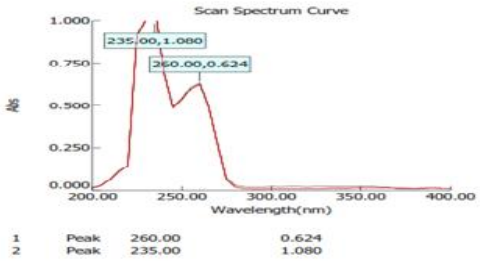

Figure 3: UV results of Valsartan

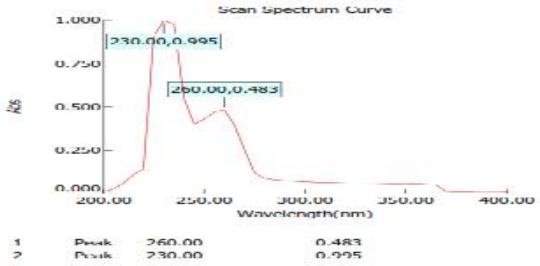

Figure 4: UV results of Sacubitril

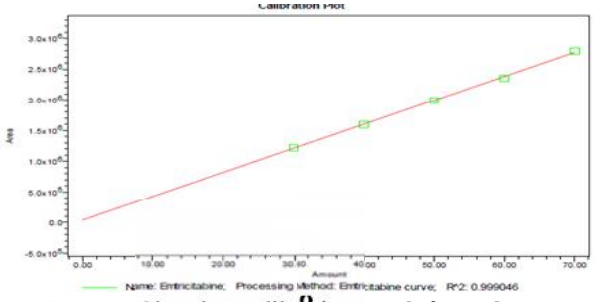

Figure 5: Showing calibration graph for Valsartan

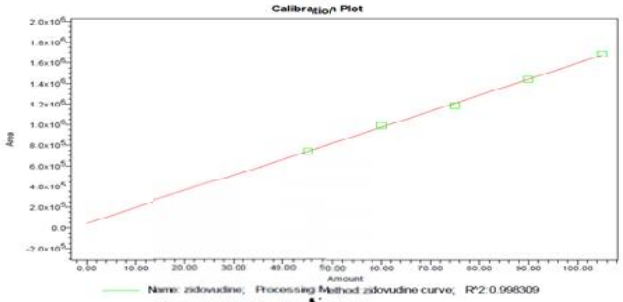

Figure 6: Showing calibration graph for Sacubitril

Method Validation

Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

Linearity:

The linearity of the peak area response was determined by making six measurements a five concentration points in the range of 20%mg/ml to 60%mg/ml of operating concentrations of standards, Valsartan and Sacubitril, Standard Area was plotted against the concentration. The linear regression coefficient, correlation coefficient, standard deviation and mean were calculated.

Accuracy:

The accuracy was assessed by using a minimum of three different concentrations of standards, Valsartan and Sacubitril, 50%mg/ml to 150%mg/ml of placebo spiked into the standard solution of Valsartan and Sacubitril, The mean, SD and RSD of accuracy were calculated.

Precision:

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

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, instruments, laboratories, assay temperatures, reagents, little variations in mobile phase, different days etc. (i.e. from analyst to analyst from, laboratory to laboratory). Acceptance criteria for ruggedness, the Percentage relative standard deviation for the area of five standard injections should not be more than 2%.

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.

Stock solution preparation:

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

Stock solution:

Further pipette 0.5ml of the above stock solution in to a 10ml volumetrick flask and dilute up to the mark with diluent.

Procedure:

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

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. The flow rate was varied at 0.8ml/min to 1.2ml/min. Standard solution 10ppm of Valsartan and 15ppm of Sacubitril was prepared and analysed using the varied flow rates along with method flow rate. The chromatograms are shown in Fig. No.40-41 and results are tabulated in Table.No.20-21. The organic composition in the mobile phase was varied from 65% to75 % standard solution 10µg/ml of Valsartan and 15µg/ml of Sacubitril were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method. The chromatograms are shown in Fig.No.42-43 and results are tabulated in Table.No.22-23. 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%.

Limit of Detection: (for Sacubitril)

Calculation of S/N Ratio:

S/N = 158/54 = 2.92

Acceptance Criteria: S/N Ratio value shall be 2.92 for LOD solution

Limit of Quantification: (for Sacubitril)

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 536/54 = 9.92

Acceptance Criteria: S/N Ratio value shall be 9.92 for LOQ solution.

Limit of Detection: (for Valsartan)

Calculation of S/N Ratio:

S/N = 165/54 = 3.05

Acceptance Criteria: S/N Ratio value shall be 3.05 for LOD solution.

Limit of Quantification:

Calculation of S/N Ratio: (for Valsartan)

Average Baseline Noise obtained from Blank: 54µ

Signal Obtained from LOQ solution: 544µV

S/N = 544/54 = 10.07

Acceptance Criteria: S/N Ratio value shall be 10.07 for LOQ solution.

Table 5: Chromatographic condition

Parameters	Description
Column	Chromosil C_{18}Column(150mm x 4.6mm)5µg.
Mobile Phase	Water: Methanol P^H 2.5 (30:70 v/v)
Flow rate	1ml min^{-1}
Wavelength	254 nm
Injection mode	Auto injector (vial)
Injection volume	20µl
RT	4.335 ,8.844

Table 6: Showing system suitability;

Name	RT	USP Tailing	USP Plate Count	USP Resolution
Sacubitril	2.605	0.2	3256	1.0
Valsartan	3.781	0.8	8542	

Table 7: Specificity results

S.No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Valsartan	2.589	2004682	342227	5167	1.3	
2	Sacubitril	3.711	1184227	162666	6389	1.2	6.5

Table 8: Linearity of Valsartan and Sacubitril

Sample ID	Valsartan		Sacubitril	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating conc.	20	1224140	10	740046
40% of operating conc.	30	1595681	15	990204
60% of operating conc.	**40***	**1992966**	**20***	**1183023**
80% of operating conc.	50	2356546	25	1439886
100% of operating conc.	60	2797214	30	1682302
Correlation Coefficient			0.999	

Table 9: Accuracy for Valsartan

Recovery level	Accuracy of Valsartan					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
150%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

Table 10: Accuracy for Sacubitril

Recovery level	Accuracy of Sacubitril					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	8.1	646754	648293.3	101.91	101.91	101.22%
	8.1	648998				
	8.1	649128				
100%	15	1172743	1174011.1	99.66	99.66	
	15	1174031				
	15	1175259				
150%	23.3	1866742	1868236.3	102.09	102.09	
	23.3	1867956				
	23.3	1870011				

Table 11: Method precision Valsartan

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	**Valsartan**	**2.586**	**2010800**	346322
2	Injection-2	**Valsartan**	**2.588**	**2002956**	340800
3	Injection-3	**Valsartan**	**2.590**	**2012800**	**346911**
4	Injection-4	**Valsartan**	**2.590**	**2005243**	**344089**

5	Injection-5	**Valsartan**	**2.591**	**2011092**	**345720**
Average				2008578.1	
Standard Deviation				4237	
%RSD				0.2	

Table 12: Method precision of Sacubitril

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Sacubitril	3.713	1184689	162348
2	Injection-2	Sacubitril	3.714	1188199	163120
3	Injection-3	Sacubitril	3.734	1195842	163500
4	Injection-4	Sacubitril	3.737	1184210	160362
5	Injection-5	Sacubitril	3.741	1198327	162484
Average				1190253.2	
Standard Deviation				6483.1	
%RSD				0.5	

Table 13: The results are summarized Valsartan

Injection	Area
Injection-1	2005053
Injection-2	2007362
Injection-3	2007473
Injection-4	2009153
Injection-5	2012800
Average	2008368.1
Standard Deviation	2874.8
%RSD	0.1

Table 14: The results are summarized Sacubitril

Injection	Area
Injection-1	1183951
Injection-2	1184689
Injection-3	1186232
Injection-4	1186406
Injection-5	1188564
Average	1185968.3
Standard Deviation	1782.3
%RSD	0.2

Table 15: Flow rate results for Valsartan

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5752	1.4
2	1.0	5026.5	1.3
3	1.2	4476	

16: Flow rate results for Sacubitril

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7187	1.2
2	1.0	6381.5	1.2
3	1.2	6471	5.0

Table 17: Showing results for Limit of Detection

Drug name	Standard deviation (σ)	Slope(s)	LOD(µg)
Valsartan	618048	39092	0.001
Sacubitril	369381	15579	0.005

Table 18: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Valsartan	618048	39092	0.004
Sacubitril	369381	15579	0.015

4. Conclusion

The results of the present study indicated that the developed method is simple, precise and cost effective for the simultaneous estimation of Valsartan and Sacubitril for routine quality control analysis of these either in bulk and pharmaceutical formulation. The developed and validated RP-HPLC method outlined is very obvious, affordable, dynamic, low cost, rapid and easy to perform with small sample volume and good repeatability. It can be adopted for the routine quality control analysis of simultaneous determination of Valsartan and Sacubitril because of good resolution of the chromatographic peaks.

5. References

[1] Skoog, West, Holler. Fundamental of Analytical chemistry. 7th edition. USA: Saunders college publishing, 1992. pp. 3-4.

[2] Synder L.R., Kirkland, J.L.Glajch, practical HPLC Method Development. John wiley and sons, INC; 2ND Edition. 1997: pp. 98-102.

[3] Marvin C.McMaster. "HPLC-A Practical User's Guide". 2nd edition. New Jersey: John Wley & sons. Inc. Hoboken-2007.

[4] SS Chitlange , Kiran Bagri and DM Sakarkar, Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. Asian J. Research Chem. 1(1): July-Sept. 2008.

[5] S. U. Kokil and M. S. Bhatia, Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan using RP HPLC. Indian J Pharm Sci. 2009 Mar-Apr; 71(2): 111–114.

[6] Gandla. Kumara Swamy N. Ravindra , S. Sowmya , A New RP-HPLC Method Development and Validation for the Simultaneous Estimation of Amlodipine and Valsartan in Tablet Dosage Forms. Asian J. Pharm. Ana. 2014; Vol. 4: Issue 3, Pg 103-107.

[7] Mustafa Çelebier, Mustafa Sinan Kaynak, Sacide Altınöz, Selma Şahin, Validated HPLC Method Development: The Simultaneous Analysis of Amlodipine and Valsartan in Samples for Liver Perfusion Studies. Hacettepe University Journal of the Faculty of Pharmacy Volume 28 / Number 1 / January 2008 / pp. 15-30.

[8] Srinath Nissankararao , Anil Kumar. A, Sravanthi. S. L. and Naga Silpa. J. Method development and validation for the estimation of valsartan in bulk and tablet dosage forms by RP-HPLC. Der Pharma Chemica, 2013, 5(2):206-211.

[9] Tamboli, A. M.; Chavan, Chetan; Mohite, S. K, Development and Validation of a RP- HPLC method for simultaneous determination of Amlodipine Besylate and Enalapril Maleate. Journal of Pharmacy Research;Nov2010, Vol. 3 Issue 11, p2564