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Analytical Method Development and Validation for the Simultaneous Estimation of Sacubitril and Valsartan by the RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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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 C18 column having dimension of (100 mm x 4.6 mm) having particle size of 5.0 µm, with mobile phase consisting of Phosphate buffer (KH2PO4 and K2HPO4) 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

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4,5]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements.

Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

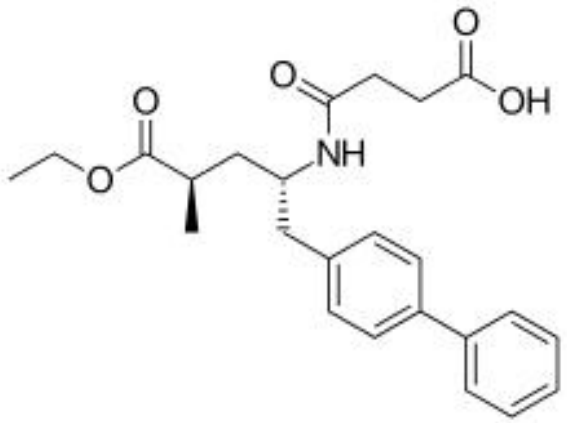

Figure 1: Sacubitril

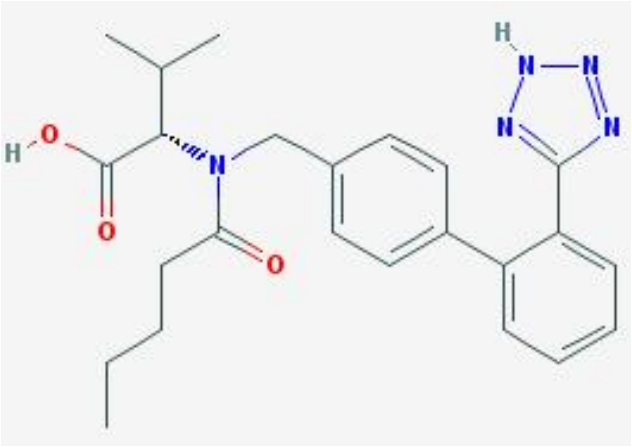

Figure 2: Valsartan

2. Materials and Methods

Apparatus: The instrument used for the study was HPLC Autosampler. Separation module2695, UV.detector2487. Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Acetonitril, Ortho phosphoric acid, Triethyl amine, Potassium dihydrogen ortho phosphate and Water [10].

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected [11]. Standard solutions of Sacubitril and Valasartan were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 254 nm was selected as the detection wavelength for the present study [12].

Selection of mobile phase

The method development and validation of Sacubitril and Valsartan 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.

Optimization Chromatographic trials for Simultaneous Estimation of Valasartan and Sacubitril by RP- HPLC.

Optimization Chromatographic conditions

Column : Thermosil C_{18} Column (100mm x 4.6mm) 5µg.
Mobile phase ratio: Phosphate buffer: Methanol P^H 2.5 (35:65 v/v)
Detection wavelength: 254 nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 2.605 and 3.781 mins

Observation:

The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

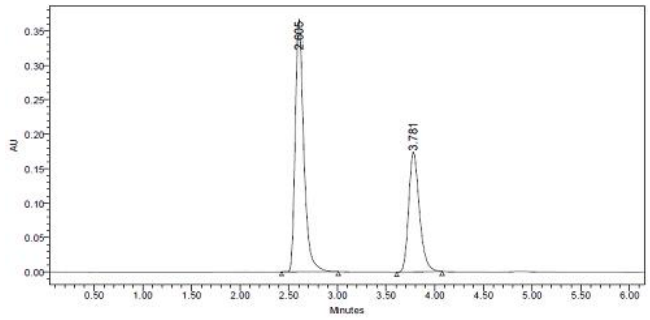

Figure 3: Optimization Chromatogram

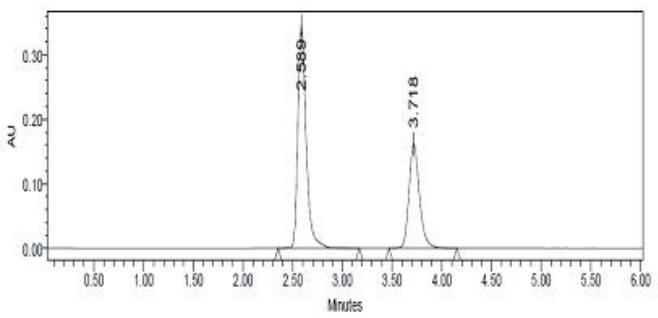

Figure 5: Chromatogram of Sample

Preparation of phosphate buffer

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 ortho phosphoric 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 [13].

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.

Valasartan and Sacbitril standard preparations

Weigh accurately 15mg Valsartan Working Reference Standard and 10mg 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)[14]. Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample solutions 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 [15]. (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 diluents [17].

3. Results and discussions

Method Validation Parameters

1. 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 [18].

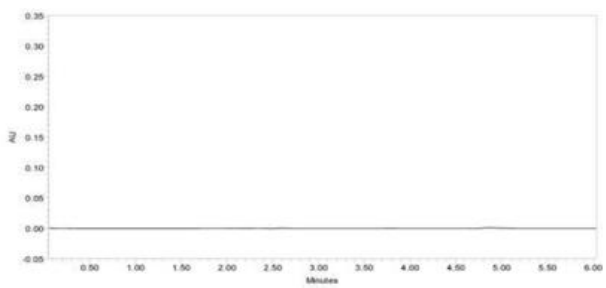

Figure 4: Chromatogram of Blank

2. Linearity and Range

Preparation of stock solution:

Weigh accurately 15mg Valsartan Working Reference Standard and 10mg 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. The solution was mixed well and used for chromatographic injection.

Valsartan:

Preparation of linearity solution (20%): 0.2ml of 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%): 0.1ml 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 (15%): 0.15ml 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 (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%): 0.3ml of 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.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

4. 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 The accuracy was assessed by using a minimum of three different concentrations of standards, Valsartan & 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.

5. Precision

Repeatability

Intermediate 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.

Intermediate Precision

Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated.

Validation of the Method

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.

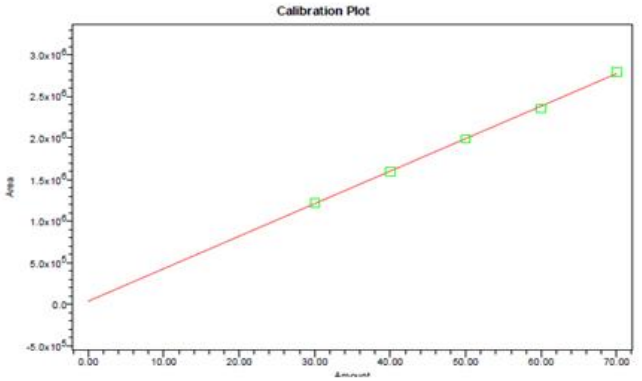

Figure 6: Calibration graph of Valasartan

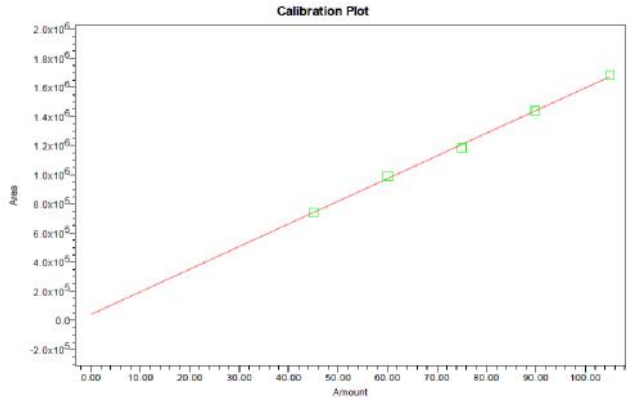

Figure 7: Calibration graph of Sacubitril

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.

Detection Limit:

The LOD was performed for Valsartan and Sacubitril was found to be 0.001 and 0.005 respectively.

Quantitation Limit

The LOQ was performed for Valsartan and Sacubitril was found to be 0.004 and 0.015 respectively.

Table 1: Calibration data of Valasartan and Sacubitril

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

Table 2: Showing Accuracy results for Valsartan

Recovery level	Accuracy of Valsartan					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	7.5	1011326	1017498.5	101.3927	101.3927	

	7.5	1015029				
	7.5	1026141				
100%	15	1986534	1987384.8	100.0106	100.0106	
	15	1987425				100.599%
	15	1988195				
150%	22.5	2989367	2992493.4	100.3936	100.3936	
	22.5	2991556				
	22.5	2996557				

Table 3: Showing accuracy results for Sacubitril

Recovery level	Accuracy of Sacubitril					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	5.0	646754	648293.3	101.91	101.91	101.22%
	5.0	648998				
	5.0	649128				
100%	10	1172743	1174011.1	99.66	99.66	
	10	1174031				
	10	1175259				
150%	15	1866742	1868236.3	102.09	102.09	
	15	1867956				
	15	1870011				

Table 4: 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	

Table 5: 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 6: Method precision data for 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 7: Method precision data for 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 8: Intermediate precision for Valasartan

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 9: Intermediate precision for 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 10: 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 11: 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 proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Valasartan and Sacubitril in tablet dosage form. 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.

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