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Research

Validated rp-hplc method for simultaneous estimation of valsartan and sacubitril in bulk and tablet dosage form.

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Valsartan and Sacubitril, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Sunfire C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (60:40% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 220nm. The retention time of the Sacubitril and Valsartan was 3.0, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Sacubitril and 75-375µg/ml of Valsartan. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Sacubitril, Valsartan, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials

with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.[1,2]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).[2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography–Mass Spectrometry), LC-MS (Liquid Chromatography–Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography–Infrared Spectroscopy), MS-MS (Mass Spectrometry–Mass Spectrometry).

HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 μm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.[6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography

In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography

In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption)

LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography)

LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions. [3.16,17]

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.[3,15]

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity.[3]

Based on elution technique
Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.
Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength. [3]

Based on the scale of operation
Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.[4]

Based on type of analysis
Qualitative analysis: Which is used to identify the compound,detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.[3]

MATERIALS AND METHODS

Scubitril-Sura labs,Valsartan-Sura labs,Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Triethylamine-Merck.

HPLC METHOD DEVELOPMENT
Trails
Preparation of standard solution
Accurately weigh and transfer 10 mg of Scubitril and Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.15ml of the Scubitril and 2.25ml of the Valsartan stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization
Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60 v/v respectively.

Optimization of Column
The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions
Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Sunfire C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (40:60v/v)
Flow rate : 0.9ml/min
Wavelength : 220nm
Injection volume : 10 µl
Run time : 6min

Validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (40:60v/v)
Column : Sunfire C18 (4.6×250mm) 5µ
Column temperature : 35ºC
Wavelength : 220nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6minutes

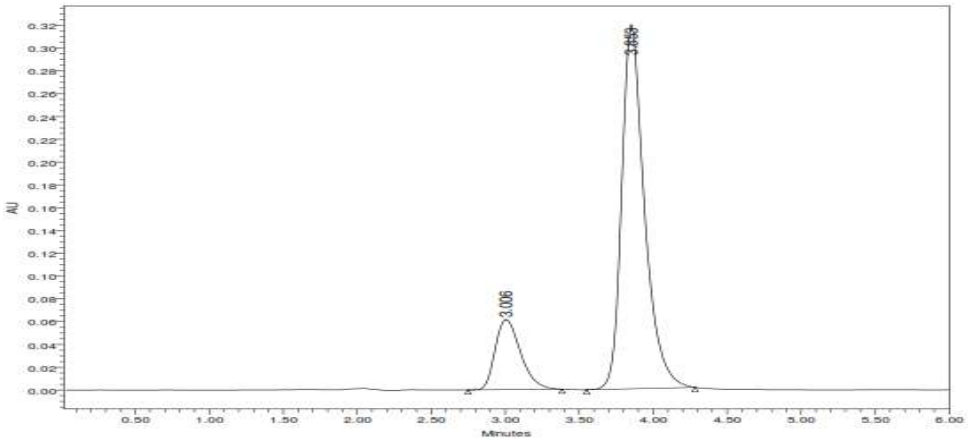


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Sacubitril	3.006	731322	61677	1.2	8574
2	Valsartan	3.853	3421257	319786	1.1	9664

Optimized Chromatogram (Sample)

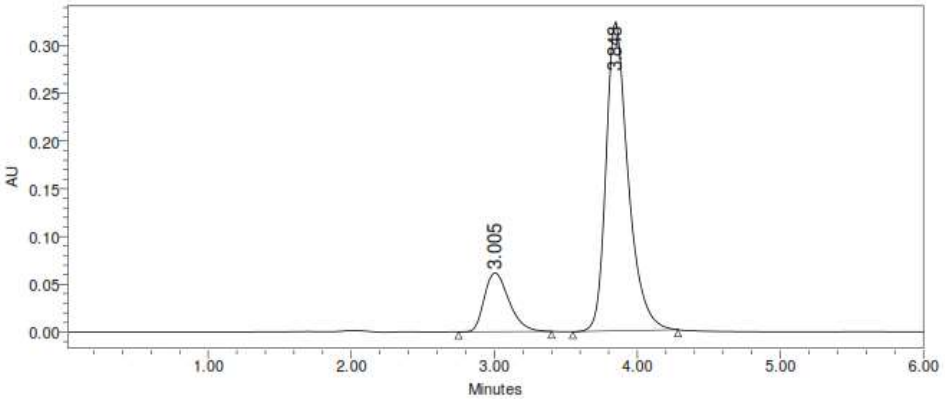


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Sacubitril	3.005	658995	61772	1.1	7442
2	Valsartan	3.848	3096188	324054	1.2	7331

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard of Sacubitril

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Sacubitril	3.008	658263	61335	7462	1.2
2	Sacubitril	3.009	658264	61947	8264	1.1
3	Sacubitril	3.008	653426	61049	6627	1.2
4	Sacubitril	3.010	653058	61141	7264	1.1
5	Sacubitril	3.006	657393	61735	6645	1.1
Mean			656080.8			
Std. Dev.			2618.946			
% RSD			0.39918			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Peak results for assay standard of Valsartan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Valsartan	3.857	3028176	381011	9583	1.1
2	Valsartan	3.859	3018373	381645	8927	1.2
3	Valsartan	3.857	3018462	381663	8465	1.1
4	Valsartan	3.861	3081711	381746	9222	1.2
5	Valsartan	3.853	3075143	381193	8462	1.1
Mean			3044373			
Std. Dev.			31427.07			
% RSD			1.0323			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample)

Table 5: Peak results for Assay sample of Sacubitril

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Sacubitril	3.008	651712	61173	1.2	8563
2	Sacubitril	3.005	657635	61936	1.1	7462
3	Sacubitril	3.007	658917	61196	1.1	9264

Table 6: Peak results for Assay sample of Valsartan

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Valsartan	3.854	3029472	361938	1.1	6476
2	Valsartan	3.853	3017462	361746	1.1	7264

3	Valsartan	3.855	3028171	371864	1.2	6545

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Sacubitril and Valsartan in pharmaceutical dosage form was found to be 100.1%

Chromatographic data for linearity study for sacubitril

Concentration	Concentration	Average
33.3	5	230247
66.6	10	462332
100	15	659905
133.3	20	892989
166.6	25	1101075

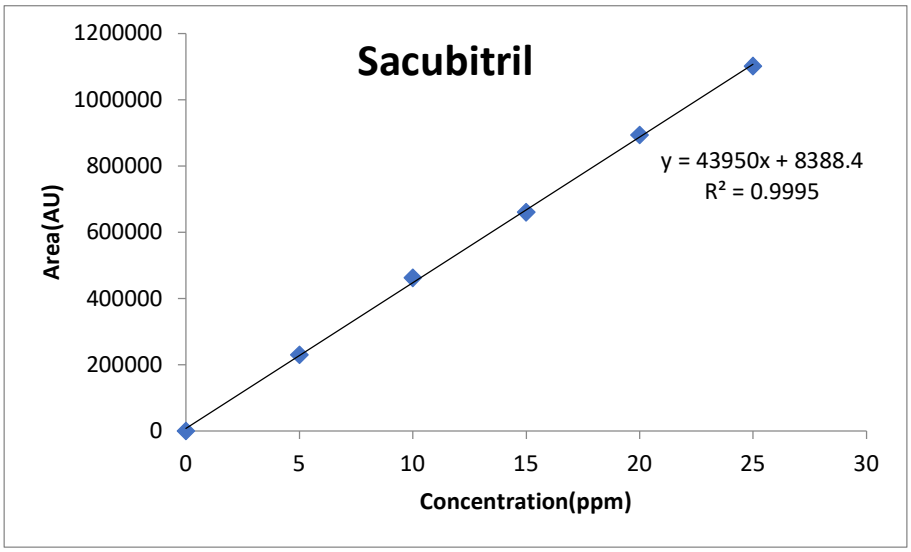


Fig 3: Chromatogram showing linearity level

Chromatographic data for linearity study for valsartan

Concentration	Concentration	Average
33.3	75	1215225
66.6	150	2135937
100	225	3020839
133.3	300	4078841
166.6	375	5058145

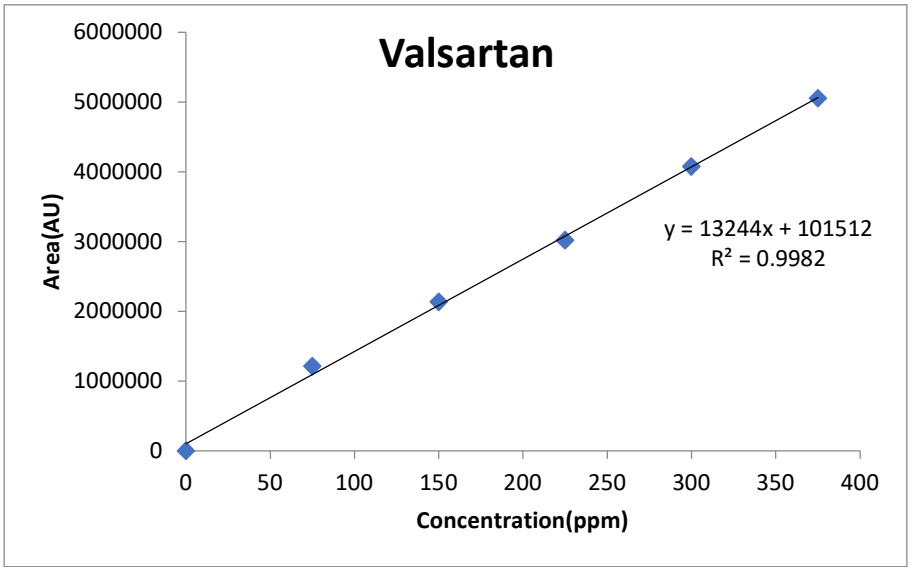


Fig 4: Chromatogram showing linearity level

Repeatability

Table 7: Results of repeatability for Sacubitril

S. No	Peak name	Retention time	Area(µV*sec)	Height	USP Plate Count	USP Tailing
1	Sacubitril	3.003	654426	61521	8474	1.1
2	Sacubitril	3.005	659862	61937	8262	1.2
3	Sacubitril	3.007	650837	62018	8117	1.1
4	Sacubitril	3.008	651433	61893	7917	1.2
5	Sacubitril	3.005	652752	61867	8011	1.1
Mean			653862			
Std.dev			3626.323			
%RSD			0.554601			

%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of repeatability for Valsartan

S. No	Peak name	Retention time	Area(µV*sec)	Height	USP Plate Count	USP Tailing
1	Valsartan	3.851	3028371	381736	6881	1.1
2	Valsartan	3.852	3009188	380138	9363	1.2
3	Valsartan	3.854	3067464	386615	7844	1.1
4	Valsartan	3.853	3076611	380183	9746	1.2
5	Valsartan	3.851	3011912	379471	7883	1.2
Mean			3038709			
Std.dev			31463.69			
%RSD			1.035429			

%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day 1:

Table 9: Results of Intermediate precision day1 for Sacubitril

S.No			Area (µV*sec)	Height (µV)		
1	Sacubitril	3.007	658911	60173	9141	1.1
2	Sacubitril	3.005	650383	61936	9662	1.2
3	Sacubitril	3.005	658813	60383	9746	1.1
4	Sacubitril	3.005	651138	60774	7746	1.1
5	Sacubitril	3.005	659937	61947	8264	1.2

6	Sacubitril	3.010	653715	61893	7836	1.1
Mean			655482.8			
Std. Dev.			4258.945			
% RSD			0.649742			

%RSD of Six different sample solutions should not more than 2

Table 10: Results of Intermediate precision day1 for Valsartan

S.No			**Area**	**Height**		
1	Valsartan	3.851	3021731	369771	8564	1.1
2	Valsartan	3.848	3019183	372746	9227	1.1
3	Valsartan	3.848	3029847	371866	7565	1.2
4	Valsartan	3.850	3028471	369017	7726	1.1
5	Valsartan	3.849	3088641	376453	6746	1.2
6	Valsartan	3.860	3056633	386621	5977	1.1
Mean			3040751			
Std. Dev.			26990.09			
% RSD			0.887613			

%RSD of Six different sample solutions should not more than 2.

Table 11: Results of Intermediate precision Day 2 for Sacubitril

S.No			**Area**	**Height**		
1	Sacubitril	3.006	648822	61847	6983	1.1
2	Sacubitril	3.008	640863	59882	7728	1.2
3	Sacubitril	3.008	643382	60774	9576	1.1
4	Sacubitril	3.007	641884	58928	8275	1.2
5	Sacubitril	3.007	647822	61483	9837	1.1
6	Sacubitril	3.005	649181	60928	8744	1.2
Mean			645325.7			
Std. Dev.			3711.009			
% RSD			0.57506			

%RSD of Six different sample solutions should not more than 2 Table:

Table 12: Results of Intermediate precision Day 2 for Valsartan

S.No			**Area (µV*sec)**	**Height (µV)**		
1	Valsartan	3.853	3075833	389911	7039	1.1
2	Valsartan	3.857	3029583	379019	9857	1.2
3	Valsartan	3.854	3021991	381875	7881	1.1
4	Valsartan	3.855	3022485	391099	7902	1.2
5	Valsartan	3.854	3085833	389222	9285	1.1
6	Valsartan	3.853	3019482	391184	8955	1.2
Mean			3042535			
Std. Dev.			30022.42			
% RSD			0.986757			

%RSD of Six different sample solutions should not more than 2

Accuracy

Table 13: The accuracy results for Sacubitril

%Concentration	**Area**	**Amount Added**	**Amount Found**	**% Recovery**	**Mean Recovery**
50%	331938	7.5	7.3	99.88	
100%	658274	15	14.7	98.89	100.166
150%	970963	22.5	22.2	101	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 14: The accuracy results for Valsartan

%Concentration	Area	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	209357	112.5	112.3	99.7%	
100%	420697.7	225	224.7	99%	99%
150%	631550.7	337.5	337.4	99%	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness -Sacubitril

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
More Flow rate of 1.0mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

Table 16: Results for Robustness-Valsartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Valsartan and Sacubitril in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Valsartan and Sacubitril was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile (60:40% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Valsartan and Sacubitril in bulk drug and in Pharmaceutical dosage forms.

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