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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN BY RP-HPLC

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ABSTRACT

A reverse phase liquid chromatographic method for estimation of Sacubitril and Valsartan in bulk drugs and marketed pharmaceutical dosage form was developed and validated. The chromatographic conditions to achieve the highest performance parameters using Targetsil C18 (4.6×150mm, 5µ) Column with guard filter were optimized. The separation was carried out using a mobile phase containing Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was taken in the ratio of 55: 45% v/v pumped at a flow rate of 1.0 mL/min with detection at 255 nm. The method was shown to be linear in 1-5µg/mL and 100-500µg/mL concentration range (regression coefficients of 0.9993 and 0.9994) for Sacubitril and Valsartan respectively. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.07µg/mL and 0.18µg/mL & 0.2µg/mL and 54.8µg/mL for Sacubitril and Valsartan respectively. The accuracy of the method was assessed by adding fixed amount of pre-analyzed sample to different standard solutions (50%, 100%, and 150% of the tested concentration) in triplicate. The percentage mean recoveries were found to 98%- 102%. The method was found to be precise with %RSD value was found to be within the limits for intraday and interday precision study, respectively. The method specificity and robustness were also established. New and sensitive RP-HPLC method for estimation of Sacubitril and Valsartan has been developed, in respect to the reviewed analytical methods.

KEYWORDS: Sacubitril and Valsartan, RP-HPLC, Accuracy, Precision, Robustness.

INTRODUCTION

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.

Inhibition of neprilysin therefore leads to reduced breakdown and increased concentration of endogenous natriuretic peptides in addition to increased levels of vasoconstricting hormones such as angiotensin II.

Valsartan is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease. It belongs to a class of medications referred to as angiotensin II receptor blockers (ARBs). It is a reasonable initial treatment for high blood pressure.

ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects, which include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation, and renal reabsorption of sodium, among others. Overall, valsartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.

EXPERIMENTAL METHODS

INSTRUMENTS USED

S.No.	Instruments and Glasswares	Model
1	HPLC	Shimadzu LC-10 AT VP with SPD-10A VP UV-Visible Detector
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil

5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra Sonicator	Labman

MATERIALS USED

S.No.	Materials	Supplier
1	Sacubitril	Azmarda
2	Valsartan	Azmarda
3	Water and Methanol for HPLC	Lichrosolv(Merck)
4	Acetonitrile for HPLC	Merck
5	Phosphate buffer	Merck

HPLC METHOD DEVELOPMENT

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Sacubitril 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 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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 Composition

The mobile phase was optimised to methanol with the proportion (100% v/v) respectively.

Optimization of Column

The method was performed with column like Targetsil C18 (4.6 x 250mm, 5um) & flow rate 1ml/min.

Optimized Method

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Sacubitril 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 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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 with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column

The method was performed with various column Targetsil 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	:Schimadzu Lc-10
ATVP Temperature	: Ambient
Mobile phase	: Methanol: Phosphate Buffer pH 3.9
(55:45v/v) Column	:C18 (4.6×150mm, 5µ)
Flow rate	: 1ml/min
Wavelength	: 284nm
Injection volume	: 10 µl
Run time	: 8 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Phosphate buffer pH 3.9

Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase

Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) 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.

METHOD VALIDATION PARAMETERS

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents

and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

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

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Sacubitril and Valsartan sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

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

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Sacubitril and 10 mg of Valsartan working standard into a 10 ml of clean dry volumetric flasks and about 7mL of Diluents and sonicate to dissolve it completely and make volume uo to the mark with the solvent. (Stock solution).

Preparation of Level – I (1µg/ml of Sacubitril & 100µg/ml of Valsartan)

Pipette out 0.01ml of Sacubitril and 1.0ml of Valsartan stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (2µg/ml of Sacubitril & 200µg/ml of Valsartan)

Pipette out 0.02ml of Sacubitril and 2.0 ml of Valsartan stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (3µg/ml of Sacubitril & 300µg/ml of Valsartan)

Pipette out 0.03ml of Sacubitril and 3.0ml of Valsartan stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (4µg/ml of Sacubitril & 400µg/ml of Valsartan)

Pipette out 0.04ml of Sacubitril and 4.0ml of Valsartan stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (5µg/ml of Sacubitril & 500µg/ml of Valsartan):

Pipette out 0.05ml of Sacubitril and 5.0ml of Valsartan stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Sacubitril and Valsartan Product Solution for Precision: Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

DAY 1

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

DAY 2

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

ACCURACY

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.015ml of Sacubitril and 1.5ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.045ml of Sacubitril and 4.5ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Sacubitril and Valsartan and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Sacubitril and 10mg of Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.03ml of Sacubitril and 3.0ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions

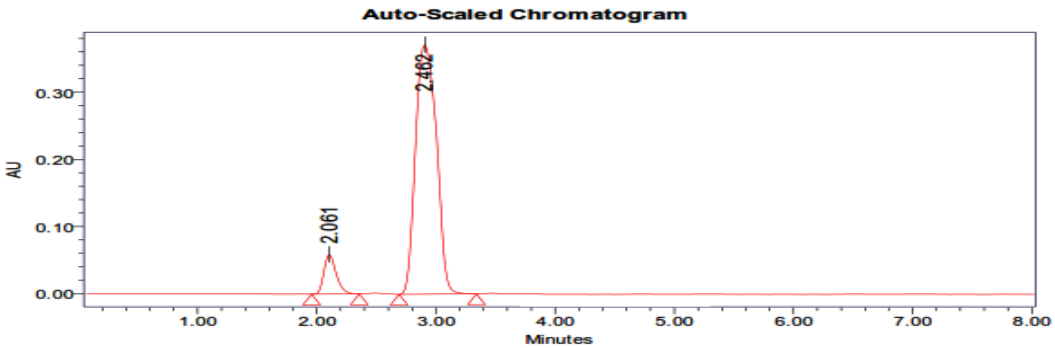
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Buffer was taken in the ratio and 50:50, 60:40 instead (55:45), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Flow rate : 1 ml/min
Wavelength : 255 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 8minutes



Optimized Chromatogram.

Peak Results For Optimized

S. No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Sacubitril	2.061	247392	58952	1.2	7243
2	Valsartan	2.462	3530866	371748	1.1	3389

Observation: From the above chromatogram it was observed that the Sacubitril and Valsartan peaks are well separated and they shows proper retention time, resolution, peak tail and plate count.So it's optimized chromatogram.

Results of System Suitability for Sacubitril

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sacubitril	2.048	246713	73455	11318	1.1
2	Sacubitril	2.074	245617	78152	7105	1.2
3	Sacubitril	2.071	245830	78146	8974	1.2
4	Sacubitril	2.069	240552	78242	7087	1.2
5	Sacubitril	2.070	245725	77705	5124	1.2
Mean			**244887.4**			
Std. Dev			**2462.26**			
% RSD			**1.005466**			

Results of System Suitability for Valsartan

S.No.	Name	Rt	Area	USP plate count	USP Tailing
1	Valsartan	2.446	3363754	8484	1.1
2	Valsartan	2.490	3326434	7889	1.0
3	Valsartan	2.489	3345949	7846	0.9
4	Valsartan	2.488	3336621	6772	0.9
5	Valsartan	2.490	3355244	6884	0.9
Mean			**3345600**		
Std. Dev			**14753.43**		
% RSD			**0.44098**		

Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Sacubitril	2.068	244102	89282	1.2	5949
2	Valsartan	2.489	3357566	576562	1.0	6866
3	Sacubitril	2.070	240052	88021	1.2	5861
4	Valsartan	2.491	3371663	576999	1.0	6808
5	Sacubitril	2.067	243230	88882	1.2	5879
6	Valsartan	2.489	3364001	570315	1.0	6823

Results of repeatability for Sacubitril

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sacubitril	2.065	249684	12079	5343	1.0
2	Sacubitril	2.064	249696	12068	5473	1.2
3	Sacubitril	2.064	246325	11949	5473	1.1
4	Sacubitril	2.065	249816	11811	5389	1.1
5	Sacubitril	2.067	249892	11735	5180	1.0
Mean			**249082.6**			
Std. Dev			**1543.964**			
% RSD			**0.61986**			

Results of Intermediate Precision for sacubitril

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sacubitril	2.066	242721	11323	5272	1.21
2	Sacubitril	2.066	240155	11564	5168	1.16
3	Sacubitril	2.066	240945	11887	5310	1.14
5	Sacubitril	2.065	240385	11938	5275	1.19
5	Sacubitril	2.069	249920	11652	5078	1.10
6	Sacubitril	2.067	240820	11750	5225	1.17
Mean			**243991**			
Std. Dev			**4641.97**			
% RSD			**1.5**			

Results of Intermediate Precision for Valsartan.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Valsartan	2.477	3325309	54143	6149	1.25
2	Valsartan	2.478	3323780	53740	6127	1.21
3	Valsartan	2.483	3328190	54791	6607	1.28
4	Valsartan	2.486	3329035	55098	6769	1.28
5	Valsartan	2.489	3325968	52379	6709	1.30
6	Valsartan	2.483	3327725	54779	6756	1.36
Mean			**3326668**			
Std. Dev			**1985.641**			
% RSD			**0.059689**			

Results of Intermediate Precision Day 2 for Sacubitril

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sacubitril	2.067	249499	11594	5240	1.2
2	Sacubitril	2.069	240991	11357	5130	1.2
3	Sacubitril	2.068	240431	11878	5136	1.2
4	Sacubitril	2.069	241330	11748	5267	1.2
5	Sacubitril	2.067	240519	11830	5222	1.2
6	Sacubitril	2.067	240470	11475	5982	1.2
Mean			**242206.7**			
Std. Dev			**3590.034**			
% RSD			**1.48222**			

Results of Intermediate Precision for Valsartan

S.NO	Name	Rt	Area	Height	USPplate count	USP Tailing
1	Valsartan	2.485	3426979	53353	6700	1.3
2	Valsartan	2.484	3446641	54454	6563	1.3
3	Valsartan	2.496	3430606	53532	6855	1.3
4	Valsartan	2.484	3430952	55157	6864	1.3
5	Valsartan	2.490	3431676	56223	6942	1.3
6	Valsartan	2.490	3429187	58578	6644	1.3
Mean			**3433812**			
Std.Dev			**7041.409**			
% RSD			**0.205061**			

The Accuracy Results for Sacubitril

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

The Accuracy Results for Valsartan

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

LIMIT OF DETECTION
Result
Sacubitril
=3.3 × 1760.8/78322
=0.07µg/ml

Valsartan
=3.3 × 61155/11150
=18.0µg/ml

LIMIT OF QUANTITATION
Result: Sacubitril
=10×1760.8/78322
= 0.2µg/ml

Valsartan
=10 × 61155/11150
= 54.8µg/ml

ROBUSTNESS
RESULTS FOR ROBUSTNESS SACUBITRIL

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

Results Of Robustness of VALSARTAN.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

CONCLUSION
This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Sacubitril and Valsartan was freely soluble in ethanol, methanol and sparingly soluble in water.
Methanol: Phosphate Buffer pH 3.9 (55:45v/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.

SUMMARY
Mobile phase is Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 8min because analyze gave peak around 2.061, 2.462 ±0.02min respectively and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 1-5µg/ml of Sacubitril and 100-500µg/ml of Valsartan of the target concentration.

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