ISSN : 2321-9602

Indo-American Journal of Agricultural and Veterinary Sciences




editor@iajavs.com
iajavs.editor@gmail.com

Indo-Am. J. Agric. & Vet. Sci., 2021

ISSN 2321–9602 www.iajavs.com
Vol. 9, Issuse 4 Oct 2021

Indo-American Journal of Agricultural and Veterinary Sciences

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF VALSARTAN AND SACUBITRIL IN TABLET DOSAGE FORM

D. Reshma Bhanu, M. G. Maddilety, S. Neelofar Sulthana

ABSTRACT

Objective: A New method was established for simultaneous estimation of Sacubitril And Valsartan by RP-HPLC method. Methods: Chromatographic separations were carried using Spurcil C18 (4.6 x 250mm, 5µm) (Dikma column) column with a mobile phase composition of 0.1% OPA buffer and Acetonitrile(50:50) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with PDA detector at wavelength 237 nm. Results: The retention time for Sacubitril and Valsartan were 3.119 and 6.851 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 12-60 µg/ml for Sacubitril and 13- 65µg/ml for Valsartan respectively. For accuracy the total recovery was found to be 99.98% and 100.28% for Sacubitril and Valsartan. The LOD and LOQ concentration for Sacubitril were found to be 0.02 µg/ml and 0.05 µg/ml and LOD and LOQ for Valsartan were found to be 0.01µg/ml and 0.04 µg/ml. The force degradation studies were performed and the results are within the limits. Conclusion: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Sacubitril and Valsartan in pharmaceutical dosage form.

Keywords ;Sacubitril,Valsartan, RP-HPLC, Validation.

INTRODUCTION

Sacubitril is an antihypertensive drug used in combination with valsartan[1].The combination drug valsartan/sacubitril, marketed under the brand name Entresto, is a treatment for heart failure[2]. Sacubitril is a prodrug that is activated to sacubitril at by de-ethylation via esterases[3]. Sacubitrilat inhibits the enzyme neprilysin. The most common adverse reactions with sacubitril plus valsartan included hypotension, hyperkalemia, cough, dizziness, and renal failure. Sacubitrilis chemically (S)-5-[(4-phenylphenyl)methyl] pyrrolidin-2-one. Structre shown in fig.1. Sacubitril Slightly soluble in water, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

Valsartan is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack[4]. Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure[5]. The drug binds to angiotensin type I receptors (AT1), working as an antagonist. This mechanism of action is different than the ACE inhibitor drugs, which block the conversion of angiotensin I to angiotensin II. Since valsartan acts at the receptor, it can provide more complete angiotensin II antagonism since angiotensin II is generated by other enzymes as well as ACE.(B) Most common side effects include dizziness, low blood pressure, and diarrhea. Valsartan is chemically (2S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazole-5-yl)biphenyl-4- yl]methyl} pentanamido) butanoic acid. Structre shown in

Pharmaceutics

Dr.K.V. Subba Reddy Institute of Pharmacy

(Approved by AICTE,P.C.l New Delhi& Permanently Affiliated to JNTUA Anantapuramu

MOU with Government General Hospital &KMC, K urnool

fig.2. Valsartan Soluble in Acetonitrile, practically insoluble in water also soluble in methanol

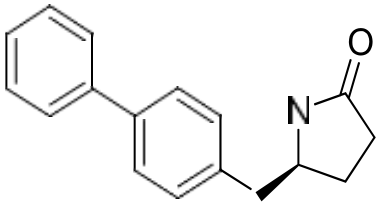

Figure 1: Structure of Sacubitril.

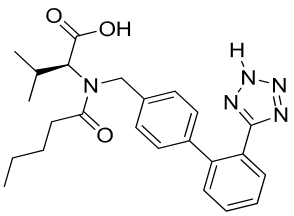

Figure 2: Structure of Valsartan.

Literature survey shows that a number of methods have been reported for estimation of Sacubitril And Valsartan individually or in combination with other drugs Those are HPLC Methods[6-16], LC-MS Methods[17], Ultraviolet spectrophotometry[18-19], However, there is only one HPLC method is reported for the simultaneous estimation of these drugs in combined dosage forms[20]. But it not explained force Degradation study.

The aim of the present study was A New Rp-Hplc Method For Simultaneous Estimation Of Sacubitril And Valsartan In Its Bulk And Tablet Dosage Form And Its Force Degadation Studies As Per Ich.

MATERIALS AND METHODS:

Chemicals and Reagents:

Sacubitril and Valsartan were obtained from Eisai pharmaceutical India pvt Ltd, Visakhapatnam. Orthophosphoric acid (Merck), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 237 nm with an Spurcil C18 (4.6 x 250mm, 5µm) (Dikma column) dimensions at ambient temperature. The optimized mobile phase consists of 0.1% OPA and Acetonitrile in the ratio of 50:50 v/v. Flow rate was maintained at 1 ml/min and run time for 16 min.

Preparation of solutions:

Preparation of buffer:

Take 1 ml of ortho phosphoric acid in 1000 ml volumetric flask and make up to the mark with HPLC water and sonicate for15minutes then then filter through 0.45µ filter under vacume filteration.

Preparation of mobile phase:

Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) degas in ultrasonic water bath for 5minutes. Filter through 0.45 µ filter under vacuum filtration

Preparation of diluent:

The Mobile phase was used as the diluent.

Preparation of standard stock solution:

Accurately weigh and transfer 12&13mg of Sacubitril & Valsartan working standard into a 10mL clean dry volumetric flaskadd Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark withdiluent.

Further pipette 3.0ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample stock solution:

Accurately weigh and transfer equivalent to 12 &13mg of Sacubitril & Valsartan s (marketed formulation=130.9 mg of tablet Powder) sample into a 10m clean dry volumetric flask add about 7 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to themark with diluent.

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

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Sacubitril & Valsartanpeaks and calculate the %Assay by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness androbustness as per ICH guidelines.

System suitability parameters:

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1ml/min for 16 minutes to equilibrate the

column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20µL of standard into Spercil C18 (4.6 x 250mm, 5µm) (Dikma column), the mobile phase of composition 0.1% OPA buffer and acetonitrile in the (50:50) was allowed to flow through the column at a flow rate of 1ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed methodare shown in table 1.

Assay of pharmaceutical formulation:

The proposed validated method was successfully applied to determine Sacubitril & Valsartan in their tablet dosage form. The result obtained for Sacubitril & Valsartan was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity and Range:

Stock solution was prepared by dissolving the appropriate amount of Sacubitril and Valsartan in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 12µg/ml to 60µg/ml of Sacubitril and 13µg/ml to 65µg/ml of Valsartan. 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. The resulte are shown in table 3.

Accuracy studies:

The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Sacubitril and Valsartan and calculate the individual recovery and mean recovery values. The resulte are shown in table 4.

Precision Studies:

Precision was caliculated from Coefficient of variance for six replicate injections of the standard. 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. The resulte are shown in table 5.

Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day. 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. The resulte are shown in table 6.

Method Precision:

To evaluate the method precision six individual samples solutions were prepared and calculate the % of Assay. The % RSD for the area of six standard injections results should not be more than 2%. The resulte are shown in table 7.

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 0.8 ml/min to 1.2ml/min. The Organic composition in the Mobile phase was varied from 45% to 55%.

LOD and LOQe sensitivity of RP-HPLC was determined from LOD and LOQ.For LOD S/N=3

For LOQ S/N=10

Where, S = Signal

N = Noise

Solution Stability:

The standard and sample solutions prepared under assay, has been kept in bench top for 24hours to perform solution stability. And inject standard and sample solutions after 24hours. The analysis performed solution stability with freshly prepared mobile phase. The % Area difference should not be more than 2%. The resulte are shown in table 8.

Force degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Sacubitril and Valsartan using the proposed method. The resulte are shown in table 9.

Preparation of stock:

Accurately weigh and transfer 12.0 mg of Sacubitril and 13.0 mg of Valsatan working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Hydrolytic degradation under acidic condition

Pipette 3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 3 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation

Ten tablets were accurately weighed and triturated it to a fine powder form and transferred into a petri dish. The petridish contained sample was subjected to thermal stress at 70 ^{0}C for about one day .The Sample solutions were prepared as equivalent concentrations of 36 µg/mL, 39 µg/mL of VAL and SAC and transferred in vials and injected in to the HPLC system.

Oxidative degradation

Pipette 3 ml above stock solution into a 10ml volumetric flask and 1ml of 12.5% w/v of hydrogen peroxide added in 10 ml ofvolumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Ten tablets were accurately weighed and triturated it to a fine powder form and transferred into a petri dish. The petridish contained sample was subjected to strees condition in the U.V chamber at 1.2 million lux for one week. The Sample solutions were prepared as equivalent concentrations of 36 µg/mL, 39 µg/mL of VAL and SAC and transferred in vials and injected in to the HPLC system.

RESULTS AND DISCUSSION

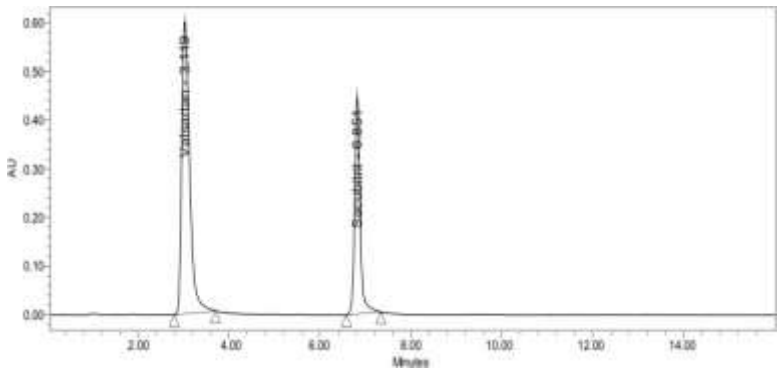

Figure 3: Standard chromatogram.

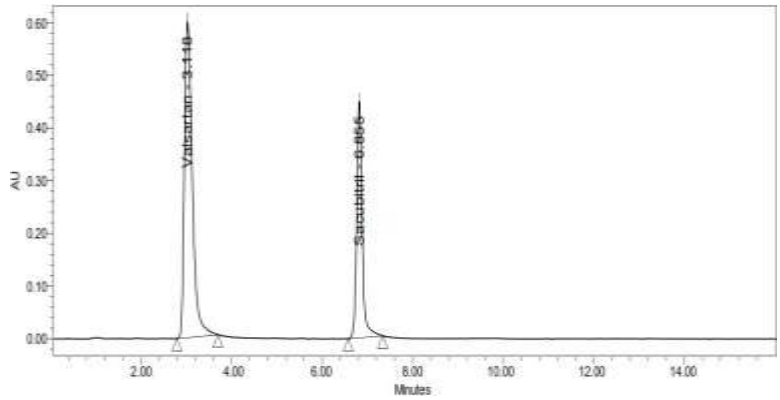

Figure 4: Sample chromatogram.

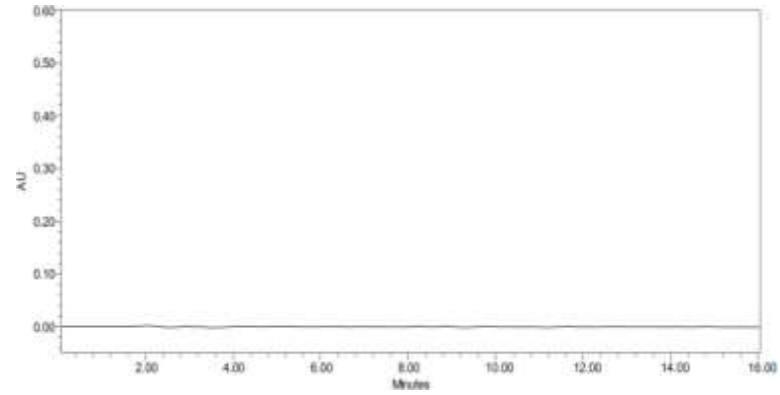

Figure 5: Blank chromatogram.

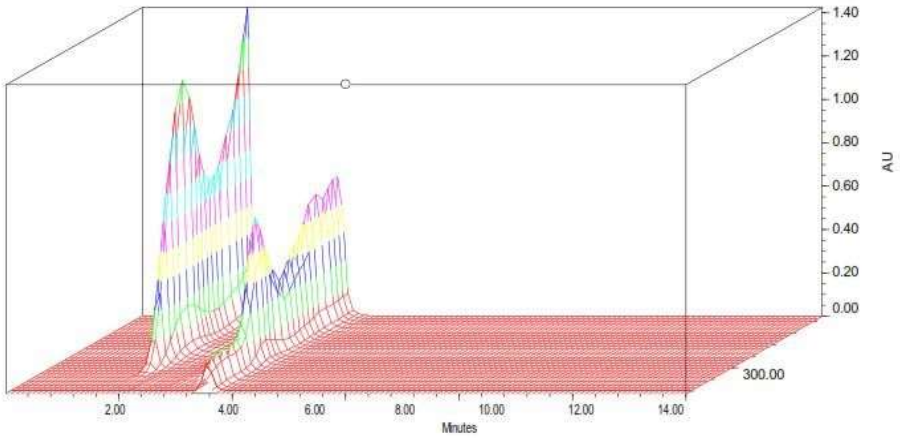

Figure 6: 3D chromatogram for standard.

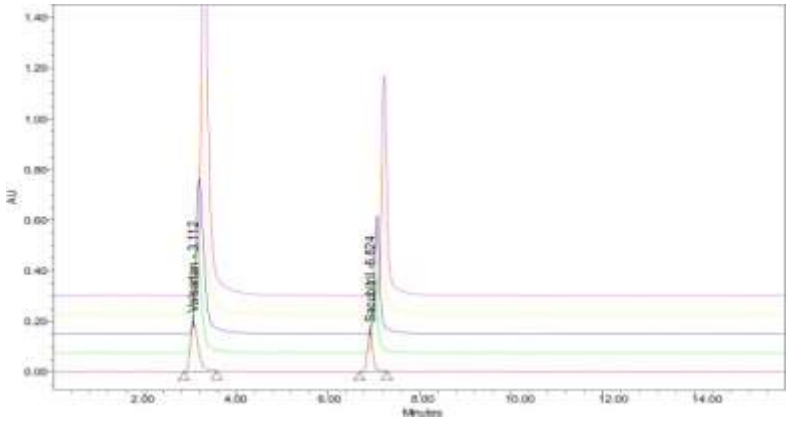

Figure 7: Linearity Overlay Chromotogram.

Table 1: System suitability parameters.

Parameters	Valsartan	Sacubitril
Retention time	3.119	6.851
USP Plate count	3558.28	17434.12
USP Tailing	1.50	1.52

Table 2: Assay results for Sacubitril and Valsartan.

	Label Claim (mg)	% Assay
Sacubitril	24	99.37
Valsartan	26	99.79

Table 3: Linearity results for Sacubitril and Valsartan.

Sacubitril		Valsartan	
Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
12	307716	13	687517
24	619777	26	1203954
36	916722	39	1849001
48	1267369	52	2447459
60	1546648	65	3012348
Correlation coefficient	0.999	Correlation coefficient	0.999

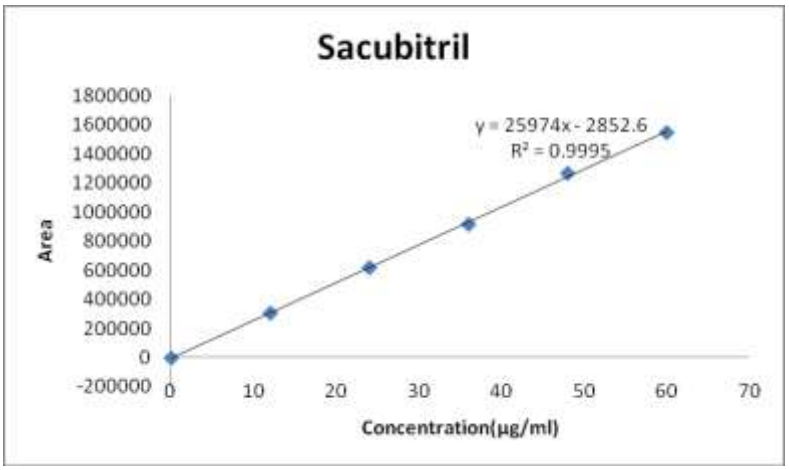

Figure 8: Linearity graph for Sacubitril.

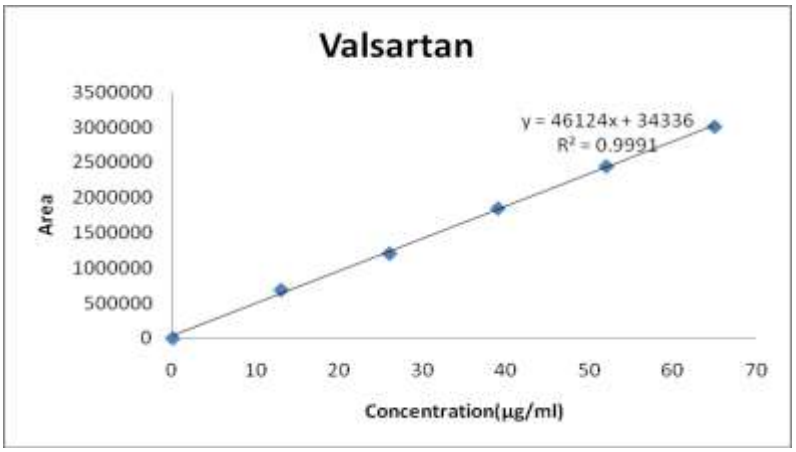

Figure 9: Linearity graph for Valsartan.

Table 4: Accuracy results for Sacubitril and Valsartan.

Drug Name	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Sacubitril	50%	465164	6	6.07	100.43	
	100%	913108	12	11.91	99.28	99.98
	150%	1382968	18	18.04	100.25	
Valsartan	50%	928676	6.5	6.50	100.03	
	100%	1858389	13	13.01	100.09	100.28
	150%	2804810	19.5	19.64	100.71	

Table 5: Precision results for Sacubitril and Valsartan.

Injection	Sacubutril Area	Valsartan Area
Injection-1	911197	1841184
Injection-2	910379	1845734
Injection-3	911492	1854819
Injection-4	911965	1865021
Injection-5	916979	1864411
Injection-6	916214	1868428
Average	913037.7	1866693
Standard Deviation	2814.9	11231.4
%RSD	0.3	0.6

Table 6: Ruggedness results for Sacubitril and Valsartan.

Injection	Sacubitril Area	Valsartan Area
Injection-1	915722	1862811
Injection-2	913937	1877531
Injection-3	919277	1870669
Injection-4	912006	1851835
Injection-5	900493	1872547
Injection-6	907901	1864770
Average	911556.0	1866693.8
Standard Deviation	6612.0	9023.0
%RSD	0.7	0.5

Table 7: Method precision results for Sacubitril and Valsartan.

Sample Name	% Assay for Sacubitril	% Assay for Valsartan
Method precision-1	99.74	100.63
Method precision-2	100.01	99.75
Method precision-3	99.55	99.27
Method precision-4	99.8	100.43
Method precision-5	99.9	100.31
Method precision-6	98.73	100.03
Average	99.62	100.07
Standard deviation	0.46	0.5
% RSD	0.46	0.5

Table 8: Solution stability results for Sacubitril and Valsartan.

S. No	Standard Area (Mean*3)	Standard area after 24hrs (Mean*3)	Sample area after 24hrs (Mean*3)	% Variation	% Assay
Sacubitril					
1	917883	915465	916168	0.26	99.39
Valsartan					
1	1853001	1845322	1846369	0.41	99.22

Table 9: Degradation results for Sacubitril and Valsartan.

Parameters	Sacubitril	% Degraded	Valsartan	% Degraded
Standard	917883.3		1853001	
Acid	896214	2.36	1738428	6.18
Base	861197	6.18	1711184	7.65
Peroxide	871965	5.00	1735021	6.37
Thermal	902006	1.73	1751835	5.46
Photo	899277	2.03	1770669	4.44

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Sacubitril and Valsartan in pure and its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Authors are thankful to the, Eisai pharmaceutical India pvt Ltd, Visakhapatnam, for providing instrumental and analytical support.

REFERENCES

1. "Sacubitril - Dictiome pronunciation database". Dictiome. Retrieved 19 April 2016.
2. John J.V. McMurray, Milton Packer, Akshay S. Desai, et al. for the PARADIGM-HF Investigators and Committees (August 30, 2014). "Angiotensin–Neprilysin Inhibition versus Enalapril in Heart Failure". N Engl J Med. 371: 993–1004. doi:10.1056/NEJMoa1409077. PMID 25176015.
3. Solomon, SD. "HFpEF in the Future: New Diagnostic Techniques and Treatments in the Pipeline". Boston. p. 48. Retrieved 2012-01-26.
4. Randa, Hilal-Dandan (2011). "Chapter 26. Renin and Angiotensin". In Brunton, L. L.; Chabner, Bruce; Knollmann, Björn C. Goodman & Gilman's The Pharmacological Basis of Therapeutics (12th ed.). New York: McGraw-Hill. ISBN 978-0-07-162442- 8.
5. Katzung, Bertram G; Trevor, Anthony J. (2015). "Chapter 11". Basic & Clinical Pharmacology (13 ed.). McGraw-Hill Education.ISBN 978-0071825054.
6. Bhole R. et al, "Development and Validation of HPLC method for Simultaneous estimation of Cilnidipine and Valsartan in bulk and tablet dosage form.", A Journal of Pharmaceutical Research 2015;6(2):28-36.
7. Siddhartha et al, "Analytical method Development and Validation for Simultaneous estimation of Nebivolol and Valsartan in bulk and pharmaceutical dosage form by RP-HPLC method.", International Journal of Pharmacy 2014;4(1):340-346.
8. Gandla Kumara Swamy et al, "A new RP-HPLC method Development and Validation for the Simultaneous estimation of Amlodipine and Valsartan in Tablet Dosage forms.", Asian Journal of Pharmaceutical Analysis 2014;4(3):103-107.
9. Parthiban C. et al, "A Validated RP-HPLC method for Simultaneous estimation of Ramipril and Valsartan in Pharmaceutical dosage form.", Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012;3(2);198-205.
10. Ramchandran et al, "Stability indicating HPLC Method for the Simultaneous determination of Valsartan and Ezetimibe in pharmaceuticals.", Tropical Journal of Pharmaceutical Research 2014;13(5):810-815.
11. Syed Sarim Imam et al, "A validated RP-HPLC method for simultaneous determination of propranolol and valsartan in bulk drug and gel formulation.", Journal of Pharm Biollied Science 2013;5(1):61-65.
12. Prasad V. et al, "A Stability indicating RP-HPLC method for simultaneous estimation of Valsartan and Atorvastatin from their combination drug product.", International Journal of Pharmaceutical Research and Analysis 2011;1(1):26-31.
13. Chitlange S. et al, "Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation.", Asian Journal Research Chem 2008;1(1):15-18.
14. Tian D. et al, "Simultaneous Determination of Valsartan and Hydrochlorothiazide in tablets by RPHPLC.", Indian journal of PharmScience 2008;70(3):372-374.
15. D. Jothiswari et al, "Validated RP-HPLC method for the Simultaneous determination of Amlodipine Besylate, Valsartan and Hydrocholorothiazide in Bulk and Pharmaceutical formulation.", Journal of Pharmaceutical and Biomedical Sciences 2010;5(6):1-7.
16. KarthikKandikattu et al,"Analytical method development and validation of simultaneous determination of Amlodipine Besylate, Valsartan and Hydrochlorthiazide in oral dosage form (tablets) by RP-HPLC technique.", Der PharmaSinica 2014;5(5):74-81.
17. Chunduri et al, "Development and validation of a reliable and rapid LC-MS/MS method for simultaneous quantification of sacubitril and valsartan in rat plasma and its application to a pharmacokinetic study." Biomedical Chromatography 2016;30(9):1467–1475.
18. Satana E, Altinay S, Gorger NG, Ozkan SA, Senturk Z. Simultaneous determination of valsartan and hydrochlorothiazide intablets by first-derivative ultraviolet spectrophotometry and LC. J Pharm Biomed Anal. 2001; 25(5-6): 1009-1013.
19. Gupta KR, Mahapatra AD, Wadodkar AR, Wadodkar SG. Simultaneous UV Spectrophotometric determination of Valsartan andAmlodipine in tablet. International Journal of ChemTech Research 2010; 2(1): 551-556.
20. Patel, K. H., S. V. Luhar, and S. B. Narkhede. "Simultaneous Estimation of Sacubitril and Valsartan in Synthetic Mixture by RP-HPLC Method." J Pharm SciBioscientific Res 2016; 6(3):262-269.