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Simultaneous estimation of sacubitril and valsartan in bulk and tablet dosage form by rp-hplc

V. Sowmya[1], A. Raja reddy[2]

[1]*Surabhi Dayakar Rao College of Pharmacy, Deparment of ANALYSIS, Rimmanaguda, Gajwel.*
[2]*C.M.R. College of Pharmacy, H.O.D Deparment of ANALYSIS, Kandlakoya, Medchal.Telangana*

***Corresponding author: V. Sowmya**

ABSTRACT

Sacubitril is used in treatment of hypertension and coronary artery disease [21a,], and valsartan used for treatment of high blood pressure, of congestive heart failure, and post- myocardialinfarction [21b]. Combination therapy in the treatment of hypertension as an appropriate treatment option is receiving boarder acceptance amongst the clinical community. Mono therapy is often not sufficient to normalize blood pressure since the goal of treatment is to normalize both systolic and diastolic blood pressure. It is proposed that these double combination tablets will be indicated as a substitution therapy in patients (i.e. patients are not to be started on this combination therapy) for the treatment of hypertension. As a replacement therapy in patients whose blood pressure is adequately controlled on Sacubitril and valsartan used as individual or combinationtherapies.Literature survey reveals the availability of some methods for estimation of Sacubitril (SBL) and valsartan (VAL) includes UV spectrometry, RP-HPLC and HPTLC alone are in combination with other drugs. Only very few HPLC estimations have been reported in the literature for the determinations of Sacubitril and valsartan present in bulk, formulations and biologicalfluids.The existing methods are inadequate to meet the requirements, hence it is proposed to improve the existing methods and to develop new methods for the simultaneous estimation of Sacubitril and valsartan in pharmaceutical dosage forms.The main objective for that is to improve the conditions and parameters, which could be easily adopted in the validation process.

Keywords: Sacubitrial,Valsartan,RP-HPLC,Validation

INTRODUCTION

Sacubitril, a prodrug and neprilysin inhibitor, used in the treatment of the patients with cardiovascular events like chronic heart failures. An ACE inhibitor or an angiotensin receptor blocker can be replaced by sacubiril in the patients with heart failure and a reduced left venticular ejection fraction (LVEF), [21]. Valsartan (trade name Diovans) is an angiotensin-II receptor antagonist, acting on the AT-1 subtype. In the U.S, valsartan is indicated for treatment of high blood pressure, of congestive heart failure, and post-myocardial infarction [21b]. In 2005, Diovanwas prescribed more than 12 million times in the United States. It is tetrazole derivative, used in treatment of hypertension.

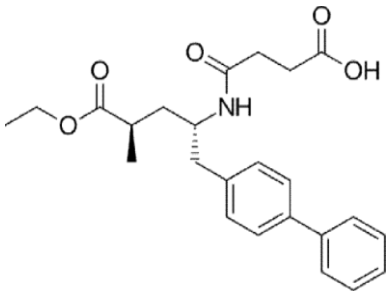

Fig 1: Structure of Sacubitril

VALSARTAN

Valsartan (trade name Diovans) is an angiotensin-II receptor antagonist, acting on the AT-1 subtype. In the U.S, valsartan is indicated for treatment of high blood pressure, of congestive heart failure, and post-myocardial infarction[21b]. In 2005, Diovanwas prescribed more than 12 million times in the United States. It is tetrazole derivative, used in treatment of hypertension.

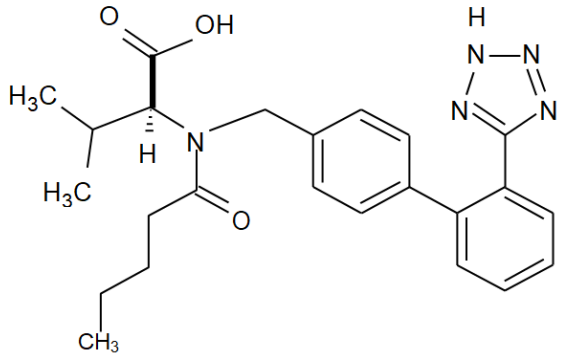

Fig 2: Chemical structure of valsartan

MATERIALS AND METHODS

S.No	Instruments And Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

S.No	Chemical	Brand names
1	SacubitrilValsaratan	Hetero labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Method development for the simultaneous estimation of Sacubitril &Valsartan

Preparation of Buffer solution

1ml of formic acid was taken into a clean in 1000ml beaker and dissolved in HPLC grade water and the volume was adjusted to 1000ml. The resulting solution was sonicated for 15min. Then the solution was filtered through 0.45µl nylon filter.

Preparation of mobile phase

The mobile phase was prepared by taking 25 volumes of buffer and 40 volumes of methanol mixed well and sonicated for 5 min. Then the solution was filtered through 0.45µl nylonfilter. Diluents: Water: Acetonitrile (50:50% v/v)

Preparation of standard stock solution

A 48.5 µg of pure Valsartan and51.5µg of Sacubitril were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 97µg/ml of valsartan and 103 µg/ml sacubitril.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 970µg of valsartan and 1030µg of sacubitril sample were weighed and transferred to 100 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluents to give a primary stock solution. From the above solution 1 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 97µg/ml of valsartan and 103µg/mlsacubitril.

Determination Of Wavelength (λ max)

10 mg of the Valsartan and Sacubitril standard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10m l volumetric flask and made upto the mark with the water to give a concentration of 10µg/ml.The above prepared solution is scanned in UV between 200-400 nm using water as blank. The $\lambda_{max}$was found to be 267nm.After several initial trails with mixtures of methanol, water, ACNand buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (25:75%v/v). The flow rate was 1.0 ml/ minute brought sharp peaks.

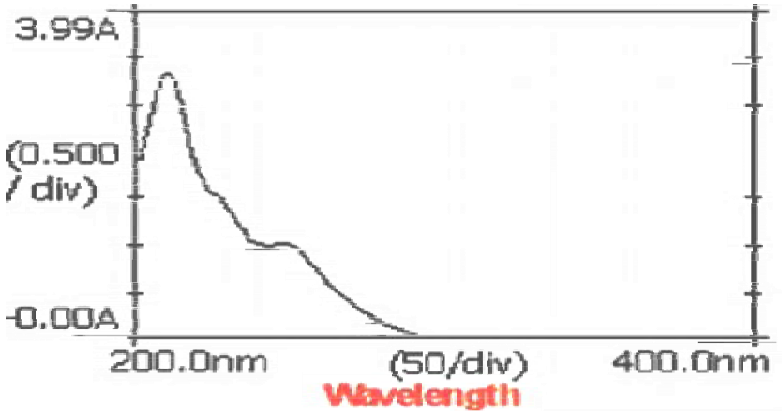

Fig 3: UV spectrum of standard Valsartan

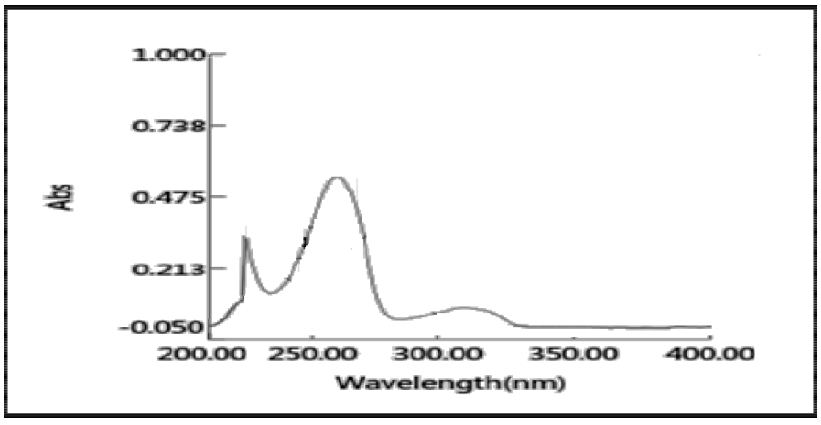

Fig 4: UV spectrum of standard Sacubitril

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 48.5 -145.5µg/ml for valsartan and 51.5 -154.5µg/ml and sacubitril. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 48.5 µg /mL, 72.75µg/mL, 97µg/mL, 121.25µg/mL and145.5µg/mL of Valsartan and 51.5g/mL, 77.25µg/mL, 103µg/mL, 128.75µg/mL and 154.5µg/mL Sacubitril

RESULTS AND DISCUSSIONS

Table 3: Linearity data of sacubitril and valsartan

S.NO	Level (%)	Sacubitil	Valsartan
		Area	Area
1.	50	1055365	581656
2.	75	11489465	8269908
3.	100	2005677	1124614
4.	125	2473826	1401252
5.	150	2923711	488699
Correlation coefficient(r^2)		0.9997	0.9997

Table 4: Method precision of sacubitril and Valsartan

S.NO	Sacubitril			Valsartan		
	RT(min)	Area	%Assay	RT(min)	Area	%Assay
injection1	3.154	2009107	99.8	2.665	112298	99.8
injection2	3.154	2001749	99.5	2.665	1125239	99.6
injection3	3.155	2007748	99.6	2.665	1126274	99.3
injection4	3.153	1998408	98.7	2.664	1125675	99.2
injection5	3.153	2006631	99.1	2.665	1125561	98.4
injection6	3.154	2004085	99.6	2.666	1124930	99.4
Mean	3.154	2004621	99.4	2.665	1125830	99.3
% RSD	0.02	0.20	0.40	0.02	0.08	0.46

Accuracy

Table 5: Recovery data of Sacubitril and Valsartan

LEVEL	S.No	% Recovery of Sacubitril	Average of Sacubitril	% Recovery of Valsartan	Average of valsartan
	1	99.4		99.7	
	2	99.7		99.2	
50%	3	100.4	99.8%	99.3	99.4%
	1	99.8		99.8	
	2	99.5		99.6	
100%	3	99.6	99.6%	99.3	99.40%
	1	99.9		99.3	
	2	99.1		98.8	
150%	3	98.7	99.2%	99.1	99.1%

Table 6: Limit of detection and limit of quantification

	Valsartan (µg)	Sacubitril (µg)
LOD	0.004	0.004
LOQ	0.012	0.012

Table 7: Results of robustness of Sacubitriland Valsartan

Parameter	RT		Theoretical plates		Asymmetry	
	SAC	VAL	SAC	VAL	SAC	VAL
Decreased flow rate (0.8ml/min)	2.635	2.229	4820	4191	1.08	1.09
Increased flow rate (1.2ml/min)	3.930	3.316	3620	3123	1.06	1.05
Wave Length 265nm	3.159	2.664	4098	3598	1.07	1.07
269	3.158	2.665	4125	3627	1.07	1.07

Table 8: System suitability data of Sacubitril and Valsartan

parameter	Sacubitril	Valsartan	Acceptance criteria
Retention time (min)	3.156	2.663	±10
Theoretical plates	4088	3598	>3000
Tailing factor	1.07	1.07	<1.50
% RSD	0.28	0.22	<2.00

Table 9: Standard Results of Sacubitril and Valsartan

S.No	Sample Name	RT (min)		Area		USP plate count		USP tailing	
		SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
1.	Injection1	3.159	2.664	2011734	1128557	4098	3598	1.07	1.07
2.	Injection 2	3.159	2.665	2014764	1130463	4125	3627	1.07	1.07
3.	Injection 3	3.156	2.662	2009811	1128528	4105	3557	1.07	1.07
4.	Injection 4	3.15	2.66	2004468	1126557	4023	3535	1.07	1.07
5.	Injection 5	3.154	2.663	2000740	1123889	4090	3597	1.07	1.07

CONCLUSION

The present experimental investigation reported in this to improve a new analytical method development and validation as per the ICH guidelines which are recommended for the analytical method validation. New analytical method developments were carried out for the simultaneous estimation of active pharmaceutical ingredients and its combined marketed dosage form. Sacubitril and Valsartan was the selected combination, marketed in the brand name Entresto (97mg of sacubitriland 103mg of valsartan). The simultaneous estimation of Valsartan and Sacubitril in drug product was done by liquid chromatography and the chromatographic separation was achieved on C18 column (X-TerraRP-18 150*4.6mm) column at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol (25:75%v/v). The flow rate was 1.0ml/ min. and ultra violet detector at 267nm. The average retention time for Valsartan and Sacubitril found to be 2.66 min and 3.154 min.

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