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Quantification of Sacubitril and Valsartan in Tablet Formulation By RP-HPLC Method

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Abstract

A simple and rapid RP-HPLC method has been developed and validated for the simultaneous quantification of sacubitril and valsartan in bulk and marketing tablet formulation. The mobile phase used for the chromatographic separation consisted of methanol and water (60:40, v/v). The separation was achieved on an Enable C18 G (250 mm x 4.6 mm x 5 µm) column using isocratic mode. Drug peaks were well separated and were detected by a UV detector at 245 nm. The method was linear in the concentration range 50-450 µg/ml for both sacubitril and valsartan. The method has been validated with respect to system suitability, specificity, precision, accuracy and robustness as per ICH guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for sacubitril were 0.8 µg/ml and 2.45 µg/ml, respectively, while LOD and LOQ for valsartan were 0.97 µg/ml and 2.95 µg/ml respectively. The proposed method was found to be simple, precise, accurate and selective and can be successfully applied for the routine analysis of sacubitril and valsartan in API and combined tablet dosage form without any interference by the excipients.

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

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1. Introduction

Valsartan(VAL) is an angiotensin II receptor commonly called angiotensin receptor blocker. Valsartan is mainly used to control high blood pressure and in the treatment of congestive heart failure. Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure. 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. Valsartan is chemically(2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenylmethyl) pentanamido] butanoic acid. Sacubitril (SAC) is categorized under antihypertensive agent , Sacubitril is a prodrug that is activated to Sacubitrilat by de-ethylation via esterase. Sacubitril inhibits the enzyme neprilysin, which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume. In addition, neprilysin degrades a variety of peptides including bradykinin, an inflammatory mediator exerting potent vasodilatory action. Sacubitril is chemically 4-[[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl) pentan-2yl]amino]-4-oxobutanoic acid. Sacubitril in combination with valsartan is used for the treatment of heart failure (Cada et al 2015 ., Dargad et al 2018., Fala et al 2015., Jhund et al 2016). Literature search reveals only few analytical methods available for determination of Sacubitril and Valsartan individually, combination and in combination with other drugs includes UV spectrophotometric(Eissa et al 2018.,Leela et al 2019.,Tatar et al 2002) , HPLC(Grace et al 2011.,Ran et al 2015., Attimarad et al 2018., Macek et al 2006., Moussa et al 2018., Zhou et al 2018 .,Kumar and Asha 2019), LC-MS(Haranadha et al 2016) and HPTLC(Ragab et al 2018) . However the reported RP-HPLC method utilizes the complex mobile phase composition , so there is a need of a simple RP-HPLC method for estimation of valsartan and sacubitril in combined dosage form. Hence, the authors attempted a simple, rapid, sensitive and accurate RP-HPLC method for simultaneous estimation of sacubitril and valsartan from API and pharmaceutical dosage form.

2. EXPERIMENTAL:

Materials and Chemicals

Sacubitril (SAC) and Valsartan (VAL) pure drugs were obtained as a gifted sample from pharma industry. Valsartan and Sacubitril tablets (ENTRESTO

TABLETS) containing Valsartan 26 mg and Sacubitril 24 mg were purchased from online pharmacies. HPLC grade water and acetonitrile was from MERCK India Ltd. HPLC grade methanol was from standard reagent Pvt Ltd Hyderabad. Nylon membrane filters 0.2 µm and 0.45 µm were from PALL life sciences Mumbai, India. Ultrasonicator used to be from LAB India Ltd Mumbai. pH meter was of Elico LI 120 make. UV Specctrophotometer was of Elico SL 210 model consisted of spectral treats software.

Instrumentation

The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 µL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0.

Chromatographic condition:

Chromatographic analysis was performed on Enable C18 G column (250 x 4.6 mm i.d, 5µ) .The mobile phase consisted of methanol and water at the ratio of 60:40 v/v. The flow rate was 1 ml/min, injection volume was 20 µL and detection was carried out at 245 nm using a UV detector.

Standard solutions

Standard stock solutions of 1000 µg/ml SAC and VAL were prepared separately by dissolving the specified weight in methanol. For the HPLC method, different working standard solutions containing 50-450 µg/ml of SAC and VAL were prepared by suitable dilutions of their respective stock solutions using the selected mobile phase as a solvent.

Analysis of tablet formulation

Twenty tablets of combined dosage form of SAC and VAL were weighed and powdered. The quantity of the powder equivalent to 100 mg of Sacubitril was transferred to 100 ml volumetric flasks. The content was mixed with methanol (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible. The solutions were then filtered through nylon membrane filter and volumes were adjusted up to the mark with methanol. An aliquot of each solution (0.5 ml) was transferred to a 10 ml volumetric flask and the volume was adjusted up to the mark with methanol to obtain the required concentration of Sacubitril (50 µg/ml) and Valsartan (54 µg/ml). The developed methods were successfully applied for the quantitative analysis of SAC and VAL in their pharmaceutical formulation using the optimized procedures mentioned before, and the analytes concentrations were accurately calculated from their corresponding regression equations.

3. RESULTS AND DISCUSSION:

Method development

In the developed HPLC method, complete base line separation, good resolution and symmetrical peaks for SAC & VAL were attained by using a green mobile phase consisting of methanol : water(60 : 40 v/v). The short separation time (10 min) and fast flow rate (1 ml/min) led to minimize the produced waste to a range of 2.0–5.0 ml/run which is considered an important measure from the greener view point. This shows that the developed method saves run time, consumed solvents and minimizes the toxic waste. This method allowed the simultaneous quantitation of the two drugs in the range of 50-450 µg/ml with UV detection at 245 nm. System suitability parameters were tested and statistically analyzed for HPLC method and indicated a good resolution of the two components

Method validation

As per ICH guidelines the developed method was validated for their system suitability, linearity, precision, accuracy, robustness, limit of detection and limit of quantification by applying the following procedures.

Linearity

The proposed methods were evaluated and validated in terms of linearity by constructing the calibration graphs within concentration ranges covering the anticipated drug concentration during the assay of the dosage form. Each concentration was analyzed in triplicates validated and summarized in Table 1..

Detection and quantitation limits

According to "ICH recommendations", the detection and quantitation limits (LOD, LOQ) of the proposed methods were determined on the basis of calculating the S.D. of the response and the slope. The theoretical values were assessed and listed in Table 2 showing that the low values of LOQ and LOD help to detect and accurately quantify low drug concentrations.

Accuracy

The accuracy of the proposed methods was ascertained via standard addition technique where different amounts of pure samples of SAC & VAL. The accuracy expressed as percentage recoveries and presented in Table 3 and 4, showed that the pharmaceutical excipients do not exert any interference. The obtained results indicated the good accuracy of the developed method.

Precision:

The precision of an analytical method was the degree of agreement among individual test results when the method was applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method was usually expressed as the

standard deviation or relative standard deviation (coefficient of variation) of series of measurement.

The components of precision, i.e., repeatability, intermediate precision and method precision in accordance with ICH guidelines, were determined as follows:

Repeatability (Intraday Variation) :

Intraday precision was checked by repeatedly injecting (n=6) mixed standard solutions of Sacubitril and Valsartan on the same day at different time intervals and % RSD was calculated. The % RSD for six replicate injections was not more than 2.0 indicates the repeatability of the method.

Intermediate precision (Inter-day Variation):

Inter-day precision checked by injecting (n=6) mixed standard solutions of Sacubitril and Valsartan on the consecutive days and % RSD was calculated. The % RSD for six replicate injections was not more than 2.0 indicates the intermediate precision of the method.

Method precision

From a homogeneous sample, the sample solutions are prepared in six replicates and % RSD was determined. The % RSD for six replicate injections was not more than 2.0 indicates the method precision.

The results of system precision and method precision for Sacubitril and Valsartan are shown in the Table 5 and 6 respectivel

Robustness:

The robustness of an analytical method was a measure of its capacity to remain un affected by small but deliberate variations in method parameters. Robustness was done by changing the mobile phase (±10%). It was observed that there were no marked changing in the chromatograms, which demonstrated that the RP – HPLC method developed is robust. % RSD for six replicate injections was determined and was not more than 2.0. The results for Sacubitril and Valsartan are shown in the Table 7.

Ruggedness:

Ruggedness was done by changing the analyst. It was observed that there were no marked changing in the chromatograms, which demonstrated that the RP – HPLC method developed is rugged. % RSD for six replicate injections was determined and was less than 2.0. The results for Sacubitril and Valsartan are shown in the Table 8.

System suitability testing

Chromatographic parameters and system suitability were validated to assure that the overall system is working properly during the analysis such as resolution, tailing factor , retention time and number of theoretical plates .The results are shown in Table 9.

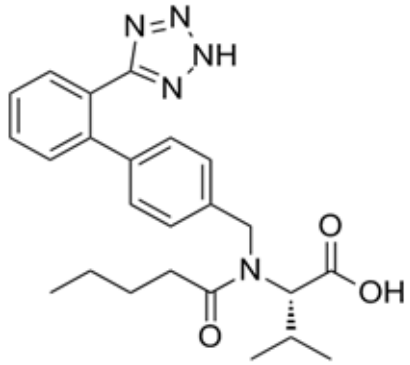

Fig 1. Structure of Valsartan

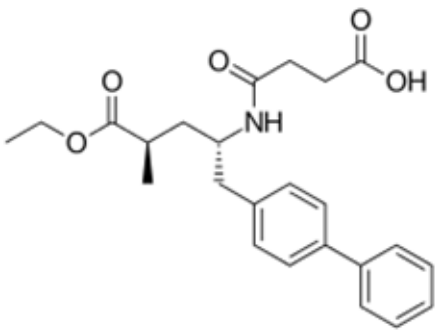

Fig 2. Structure of Sacubitril

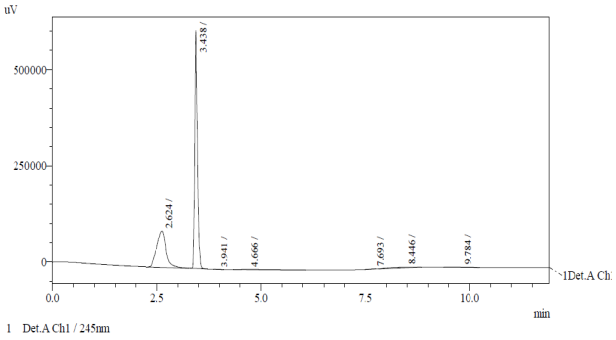

Fig 3 : Chromatogram of Standard Solution of Sacubitril and Valsartan

Table 1: Calibration data for Sacubitril and Valsartan

Linearity Conc (µg/ml)	Sacubitril Peak area	Valsartan Peak area
50	69642	56405
100	135885	115408
150	201092	167941
200	263656	224495
250	327544	285076
300	395506	341254
350	454346	398614
400	522930	455787
450	596429	505643

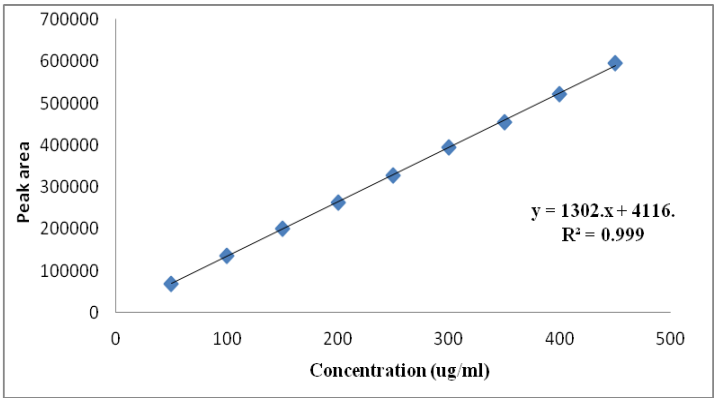

Fig 4: Calibration curve for Sacubitril

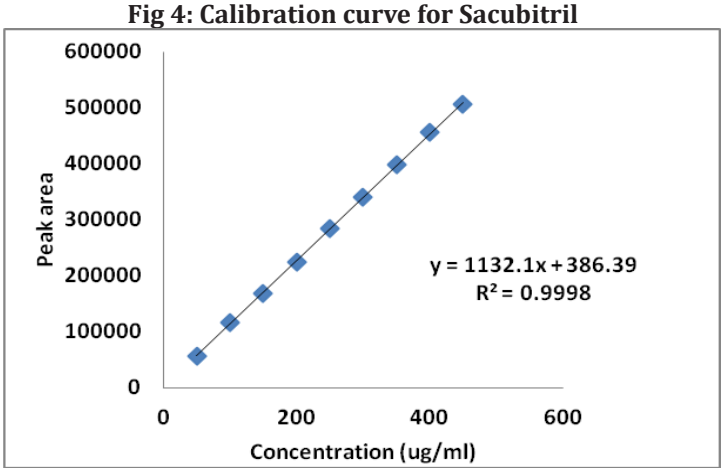

Fig 5: Calibration curve for Valsartan

Table 2: LOD and LOQ data for Sacubitril and Valsartan

Drug	LOD (µg/ml)	LOQ (µg/ml)
Sacubitril	1.166	3.533
Valsartan	1.024	3.104

Table 3: Recovery studies of Sacubitril

Spiked level (%)	Actual conc (µg/ml	Conc. added (µg/ml)	Conc. found (µg/ml)	Percent recovery (% w/w)	Mean % recovery	% RSD
80	100	80	79.98	99.81	99.97	0.61
80	100	80	79.54	100.65		
80	100	80	79.76	99.45		
100	100	100	99.85	100.77	99.93	0.78
100	100	100	98.78	99.23		
100	100	100	99.85	99.79		
120	100	120	119.78	100.35	100.18	0.93
120	100	120	119.84	99.17		
120	100	120	119.96	101.02		

Table 4: Recovery studies of Valsartan

Spiked level (%)	Actual conc (µg/ml)	Conc. added (µg/ml)	Conc. found (µg/ml)	Percent recovery (% w/w)	Mean % recovery	% RSD
80	104	83	82.83	100.11	100	0.76
80	104	83	82.64	99.20		
80	104	83	82.34	100.71		
100	104	104	103.76	99.09	99.99	0.81
100	104	104	103.83	100.19		
100	104	104	103.42	100.69		
120	104	124	122.86	100.14	100.7	0.61
120	104	124	123.54	100.59		
120	104	124	123.84	101.37		

Table 5: System Precision data of Sacubitril and Valsartan

S.no	Sacubitril		Valsartan	
	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
1	250	327544	250	285076
2	250	329276	250	289682
3	250	329453	250	289956
4	250	328356	250	288653
5	250	325289	250	284927
6	250	326720	250	286554
Mean	327773		287475	
S.D	1429.17		2258.74	
% RSD	0.43		0.78	

Table 6: Method Precision data of Sacubitril and Valsartan

Injections	Intra-day Precision		Inter-day Precision	
	Sacubitril Peak area	Sacubitril Peak area	Valsartan Peak area	Peak area
1	328620	152003	334634	156113
2	332145	155143	334997	156027
3	332896	154278	328620	152003
4	334972	153911	332143	155846
5	326198	151989	326592	151889
6	325681	151860	329867	155632
Mean	330085	153197	331397	154376
SD	3812.56	1423.92	3701.16	2220.36
% RSD	1.15	0.92	1.11	1.43

Table 7: Robustness studies of Sacubitril and Valsartan

S. No	Sacubitril			Valsartan		
	Changing in mobile phase (±10%)			Changing in mobile phase (±10%)		
	Methanol : Water			Methanol : Water		
	50:50	50:45	45:55	50:50	50:45	45:55
1	340341	338263	341589	153669	153182	154055
2	344162	342355	345292	157236	145293	153928
3	344069	342917	345063	157569	158328	158263
4	343816	334842	344592	149882	156912	148241
5	339413	334842	339817	156152	150636	156725
6	335189	337248	338252	147178	151253	145839
Mean	341165	339578.3	342434.16	153614.3	152600.6	152841.8
SD		1121.94	897.43		716.77	546.23
% RSD		0.32	0.26		0.46	0.35

Table 8: Ruggedness studies of Sacubitril and Valsartan

S. No	Sacubitril		Valsartan	
	Change of analyst		Change of analyst	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	340341	338819	153669	152593
2	344162	342695	157236	154839
3	344069	344928	157569	142933
4	343816	334084	149882	154796
5	339413	339182	156152	158921
6	335189	341556	147178	148423
Mean	341165	340210.7	153614.3	152084.2
SD		674.79		1081.96
% RSD		0.19		0.7

Table 9:- System Suitability Parameters

Parameters	SAC ± SD (n=6)	VAL ± RSD (n=6)
Retention Time (min)	2.624 ± 0.7154	3.428 ± 0.4430
Tailing factor	0.953 ± 0.924	1.361 ± 0.966
Theoretical Plates	9230 ± 0.823	8556 ± 0.246
Resolution	6.42 ± 0.56	

Table 10. Analysis of Sacubitril and Valsartan in commercial formulation

Formulation	Labelled claim(mg)		Amount found*(mg)		%Recovery*±%RSD	
	Valsartan	Sacubitril	Valsartan	Sacubitril	Valsartan	Sacubitril
Entresto Tablets	26	24	25.93	23.84	99.73±0.34	99.33±0.26

CONCLUSION:

The proposed HPLC method could be considered as selective, and sensitive methods for the quantitative simultaneous determination of SAC and VAL, which represent an advantage over the previously published methods. Based on isocratic elution, the proposed HPLC method provided high selectivity and sensitivity for the determination of SAC and VAL in short analysis time at single wavelength. The proposed HPLC method was found to be more sensitive with high linearity range and low LOD and LOQ values when compared with the reported methods.

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