Research Article

SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A new and simpler method was developed with the Inertsil ODS 3V C18 column, linked to a C_{18} guard cartridge and PDA detection at 239 nm, to quantitably detect Sacubitril (SAC) and Valsartan (VAL). The optimized mobile phase acetonitrile (ACN), triethylamine buffer (TEA) was pumped at 0.7 mL/min. The method was linear between 5-15 µg/mL for SAC and 2.5-7.5 µg/mL for VAL, statistically validated for its accuracy, precision, and linearity. The additives in the commercial tablet were found to have no interference in the method. The currently developed method for estimating compounds SAC and VAL associated with the tablet dosage form can be used routinely.

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INTRODUCTION

Sacubitril (SAC) (**Fig.1)**, chemically 3-{[(2S,4R)-1-{[1,1'-biphenyl]-4-yl}-5-ethoxy-4-methyl-5-oxopentan-2-yl]carbamoyl}propanoic acid. SAC is a white powder. The solubility of drug substance is Sparingly soluble in methanol,ethanol & acetonitrile. SACis a prodrug neprilysin inhibitor and is used in combination with VALis used to reduce cardiovascular event risk in chronic heart failure patients.

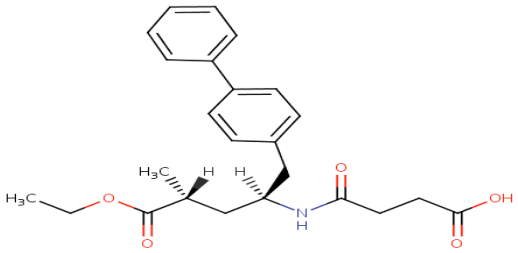

Fig 1: Structure of SAC

Valsartan (VAL), chemically (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)pentanamido]butanoic acid as shown in Fig 2.

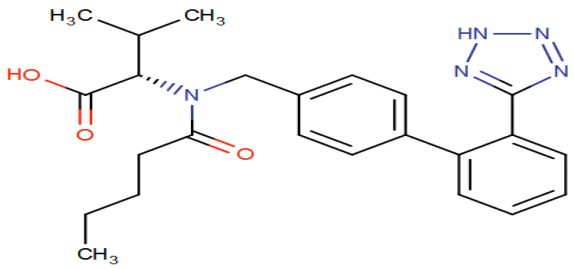

Fig 2: Structure of VAL

VAL is a White crystalline powder. VAL is freely soluble in alcohol,ether & chloroform. Literature review shows that some analytical procedures have been taken into account for estimation of SAC and VAL individually as stability indicating and in biological fluids or in combination with different drugs in pharmaceutical dosage forms.

For the simultaneous identification of SAC and VAL, both pharmaceutical dosage and biological fluids, all monotonous or expensive techniques, have been considered since the late HPLC [1-2] and UV spectroscopy [3].

MATERIALS AND METHODS

Chromatographic measurements were made on an RP-HPLC Shimadzu (LC 20 AT VP) model which consisted of an LC-20AD solvent delivery module, SPD-M20A prominence diode array detector, a rheodyne injector (model 7125, USA) valve fitted with a 20µl loop.The machine was operated using a SCL-10A system controller and a personal computer with chromatographically mounted Shimadzu software (LC Solution, Published 1-11SP1). Branson Sonicator was used to degass the mobile process (Branson Ultrasonic Corporation, USA). A 1 cm long quartz cell for spectrum recording was used with a UV double-beam spectrophotometric system (Systronices 2202 Mode UV-1601PC).

Chemicals and reagents

Working standards of SAC and VAL were purchased from Biotech Solutions, New Delhi. ACN, MeOH of HPLC grade and HCOOH was of analytical- reagent grade supplied by M/S SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The pharmaceuticals entresto (Batch No: 659913A, MFG: APR 2018, EXP: APR 2021) were purchased from local medical shop.

Chromatographic condition

Chromatographic separations were carried out on an Inertsil ODS C_{18}column (250 mm× 4.6mmi.d., 5µm) connected with an Inertsil C_{18} guard cartridge. The mobile phase consisted of TEA : ACN (pH 3.5 ±0.5) (50:50). A wavelength of 239 nm was selected for detection. The sample was injected at 20µl. The HPLC method was used in the laboratory setting (20±2 °c)

Preparation of Standard Solutions

In the mobile phase, SAC and VAL stock standard solutions were drawn up to prepare 1mg/ml solution. The ready to use stock arrangements were then shielded from light at 4°C ± 0.05. During analysis day,the working standard solutions were freshly arranged by diluting the stock solutions with mobile phase.

Selection of detection wavelength

At 239 nm, SAC and VEL shown significant absorbance by PDA detector.

Preparation of Sample Solution

Ten tablets were weighed (each tablet entresto containing SAC-24mg and VAL-26mg and taken into a mortar and crushed to fine powder and uniformly mixed. In order to achieve weight equal to 10 mg SAC and 15 mg VAL, the tablet stocking solutions of SAC / VAL (µg / ml) were produced and dissolved in a mobile phase enough. A 0.45-micron syringe filter was then screened and sonicated for 5 minutes. The addition of 1.5 ml of stock solution to 10 ml of the mobile process is used in preparing the further dilutions in 5 replicates 100µg / mL of SAC, and in 150µg / ml of VAL.

Assay validation

The RP-HPLC method, optimized has been accepted for various parameters [4] in accordance with the guidelines [ICH] Q2 [R1].

Linearity and range

For linearity a concentration ranges from 5 to 15 µg/mL ofSAC and 2.5 to 7.5 µg/mL of VAL was prepared. By taking the peak area versus the concentration, a calibration graph was drawn. A study was conducted for correlation coefficient, intercept, slope and linear regression [5].

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) were determined respectively with formulas 3.3 s / S and 10 s / S, where s is the standard deviation from response (y-intercept) and S the slope of linearity plot was calculated[6].

Specificity

The specificity was determined by comparing sample solution analysis with findings from standard pharmaceuticals. [7].

Precision studies

Precision was calculated by taking 10 µg/mL concentration of SAC and 6.25 µg/mL of VAL sample ware analyzed six times on a similar day to find out any differences in the results [8].

Accuracy studies

Accuracy is the closeness in accordance between the accepted true value and the actual results obtained. Accuracy studies are typically measured by measuring the regeneration of a spiked analyte sample in the sample mixture to be tested. For accuracy studies, three different concentration of solution such as 50%, 100% and 150% were used. After injecting each concentration mean % recovery was calculated [9].

RESULTS AND DISCUSSION

Chromatographic development

Chromatographic analysis was developed using an Inertsil ODS C_{18} column (100mm× 4.6 mmi.d., 5µm). The mobile phase consists of TEA : ACN (pH 3.5 ±0.5) (50:50) and that was supplied at a flow rate of 0.7 mL/min. A wavelength of 239 nm was selected for detection. Fig. 3 shows the optimized chromatogram of SAC and VAL.

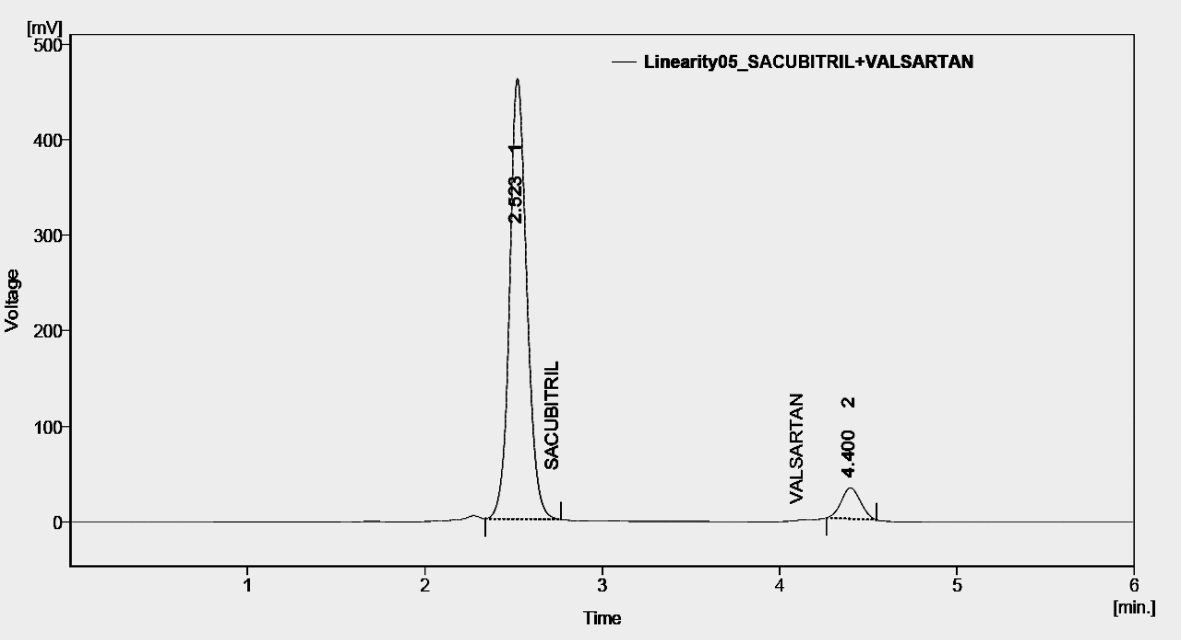

Fig. 3: Optimized chromatogram of SAC and VAL

Validation of the method

Linearity

The response from the analytes in the 5-15 µg / mL of SAC and 2.5-7.5 µg/mL of VAL range was linear (r^2=0.999). The results wereshown in Table 1. Fig. 4 and Fig. 5 shows the calibration curve.

Table 1: Linear regression analysis of SAC and VAL

Parameters	SAC	VAL
Linearity range (µg/mL)	5–15 µg/mL	2.5 to 7.5 µg/mL
Correlation Coefficient (r^2)	0.999	0.999
Slope	205.4	30.36
Intercept	5.604	22.47

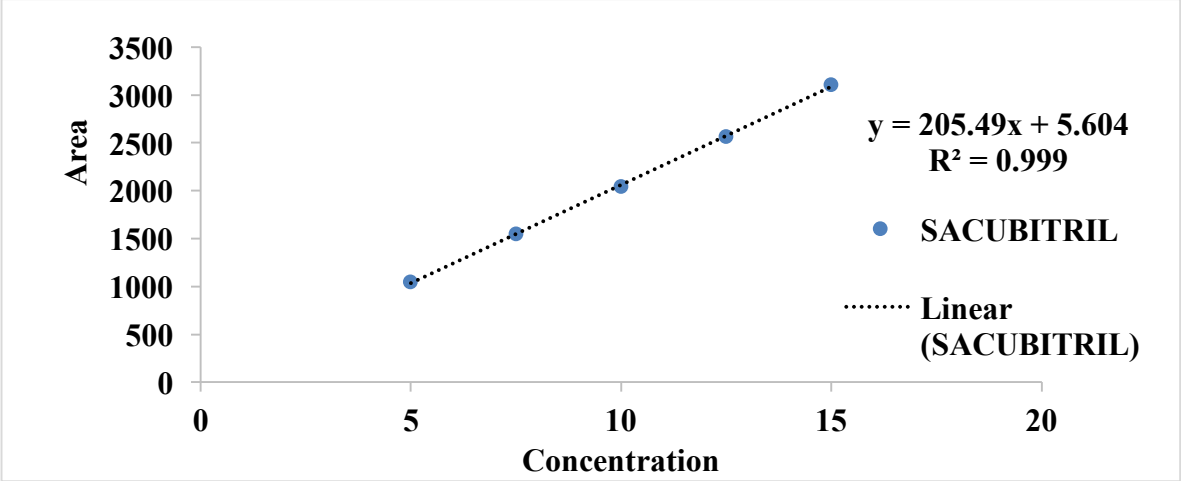

Fig. 4: Linearity graph of SAC

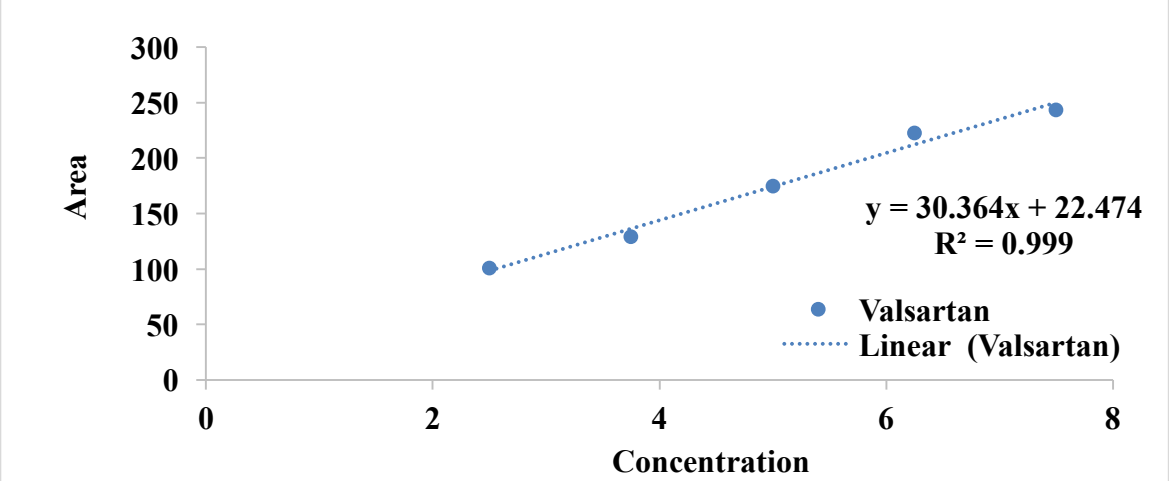

Fig. 5: Linearity graph of VAL

The selected concentration gives acceptable accuracy and precision over a wide concentration range. The resultsdemonstrate that an excellent correlation coefficient between theabsorbance and concentration of SAC and VAL.

Sensitivity

The LOD was found to be 0.015 µg and 0.038µg for SAC and VAL respectively. The LOQ for SAC and VAL were found to be 0.004µg and 0.011µg respectively representing good sensitivity of the method.

Specificity

The standard and sample solutions obtained chromatograms do not conflict withwhich indicates method is highly selective.

Precision

In the estimation of SAC and VAL calculations (Table 2) have shown that the RSD level during the study has been < 2%. These low RSD values show that the precision is good.

Table 2: Precision studies of SAC and VAL

Drug	Actual Concentration	Precision Data	% RSD
SAC	10 µg	99.99	0.0333
VAL	6.25 µg	99.99	0.0254

Accuracy

The accuracy study reveals influences on quantitative parameters of additives that are typically present in dosage forms. The recovery study data provided in Table 3 shows that more than 99% of the accurativity of quantifying SAC and VAL products is reliable to estimate commercialized formulation used in the study indicates that the proposed simultaneous HPLC-RP method is reliable.

Table 3: Results of recovery studies of SAC and VAL

Amount taken (µg/mL)		Amount added (µg/mL)		% recovery		%RSD	
SAC	VAL	SAC	VAL	SAC	VAL	SAC	VAL
10	5	5	2.5	96.33	100.56	0.003	0.005
10	5	10	5	101.67	100.87	0.007	0.003
10	5	15	7.5	100.65	100.31	0.005	0.004

ANALYSIS OF A MARKETED PREPARATION

The results obtained for the amount of SAC and VAL in tabletpowder against the label claims werein good agreement it indicates that there is no interference fromthe excipients presents in the tablet. The percent

assay was foundto be 101.67% and 100.87%, for SAC and VAL respectively.

CONCLUSIONS

The plan to conduct this research work is to create and approve amethod utilizing a simple, rapid, sensitive, precise, and accurateRP-HPLC for the routine determination of SAC and VAL inbulkand pharmaceutical preparations. The proposed method is appropriate for pharmaceutical investigation indifferent analytical laboratories. The retention time and run timewere short, hence, requires less mobile phase for this technique,making it more economical and rapid. Consequently thetechnique can be utilized for the analysis of a large number ofsamples.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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