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Simultaneous Estimation of Sacubitril and Valsartan Combination of Drug in Tablet Dosage Form Using Hydrotropy by UV Spectrophotometry

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Abstract: Sacubitril/valsartan, traded under the brand name Entresto between others, is a fixed-dose combination medication for use heart failure. Sacubitril is a neprilysin inhibitor (A prodrug) and is used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure. It is anti - hypertensive drug. Valsartan is an Angiotensin Receptor Blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Two UV-spectrophotometric methods have been developed and validated for simultaneous estimation of Sacubitril and Valsartan in a tablet dosage form. The first method employed solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 226.0 nm and 254.0 nm, λmax for Sacubitril and Valsartan, respectively. The second method was absorbance ratio method, which involves formation of *Q*-absorbance equation at 240 nm (isoabsorptive point) and also at 254 nm (λmax of Valsartan). The methods were found to be linear between the range of 4-12 μg/mL for Sacubitril and 2-10 μg/mL for Valsartan using Methanol as solvent. The mean percentage recovery was found to be 96.68%and 101.89% for the simultaneous equation method and 100.2% and 104.53% for the absorbance ratio method, for sacubitril and valsartan respectively. It could be concluded from the results obtained in the present investigation that the two methods for simultaneous estimation of sacubitril and valsartan in tablet dosage form are simple, rapid, accurate, precise and economical and can be used, successfully, in the quality control of pharmaceutical formulations and other routine laboratory analysis. The reviewed highlights various analytical techniques such as high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), UV Spectroscopy, high per-formance thin layer chromatography (HPTLC), liquid chromatography coupled to tandem mass spectrometry (LC- MS), RP-HPLC and other chromatographic method used. The combination of these drugs with different method was examine and the commonly use of the drugs in hypertensive.

Keywords: Sacubitril, Valsartan, Distilled water, Methenol, Simultaneous Estimation, Hydrotropy H UV Spectrophotometry, ICH Guidelines

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INTRODUCTION

Analytical chemistry is a branch of science which is useful in various fields of science in pharmaceuticals and medicine due to its versatile application. It deals with two aspects of chemical analysis i.e. qualitative and quantitative analysis.

Spectrophotometric method

It is the bough of science commerce with the study of interaction between electromagnetic radiation and matter. It is a most powerful device available for the study of atomic and molecular structures and is used in the analysis of wide range of samples. Some of the commonly used Spectrophotometric methods are as follows,

- Simultaneous equation method (Vierdott"s method)
- Derivative Spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Solvent extraction method
- Dual wavelength method
- Geometric correction method
- Orthogonal poly nominal method
- H-point standard addition method
- Least square approximation method

- **Simultaneous equation method**

If a sample contain two absorbing drugs (X & Y) each of this absorbs at the λ_{max} of each other i.e. λ_1 and λ_2, it may be possible to determine both the drugs by the technique of simultaneous equation method provided that certain criteria apply.

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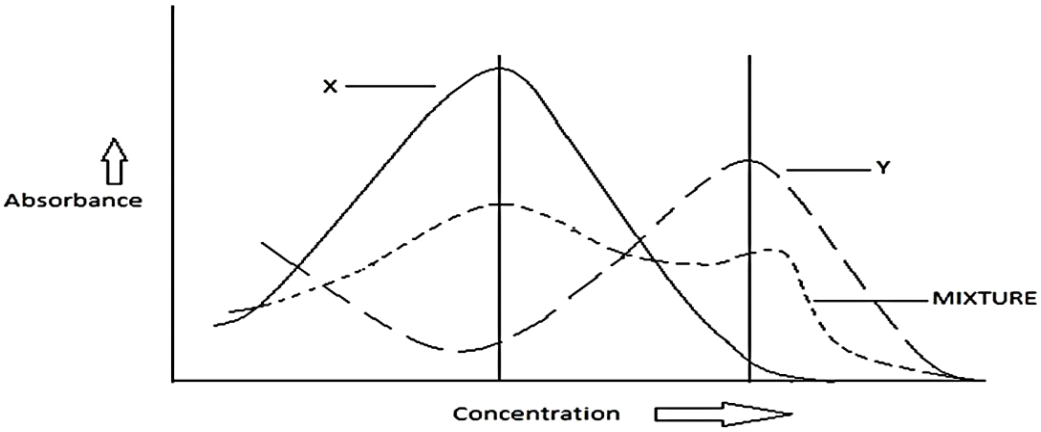

Figure 1: Overtone spectra of substance X and Y

- The information required is:
- The absorptivity of X at λ_1 and λ_2 and ax_1 and ax_2 respectively.
- The absorptivity of Y at λ_1 and λ_2 and ay_1 and ay_2 respectively.
- The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.
- Let C_x & C_y be the concentration of X & Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance of X & Y.

At $\lambda 1A1 = ax1\ bcx + ay1\ bcy$

At $\lambda 2A2 = ax2\ bcx + ay2\ bcy$

On rearranging equation (2)

$$cy = A2 - ax2cx / ay2 \quad (3)$$

Substitution in equation (1) and rearranging, gives:

$$cx = A2ay1 - A1ay2 / ax2ay1 - ax1ay2 \quad (4)$$

And

$$cy = A1ax2 - A2ax1 / ax2ay1 - ax1ay2 \quad (5)$$

The ratios are:

$$A2/A1 /ax2ax1 \ \& \ ay2/ay1 / A2/A1$$

there is using two equations the concentration of component X and component Y in the mixture of sample which can be determined.

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- **Hydrotropy Theory**

Hydrotropy is the term discovered by „Carl A. Neuberg" in 1916. Now days the hydrotropic solution possess high industrial demand due to their exclusive features like better solubility, absence of fire hazards, good recovery and fast separation feature without any emulsification problem. It produces eco-friendly nature and effective water solubility. It involves the water-soluble or water-insoluble categories. Most of the newly developed drug molecules are lipophilic in nature and pitiable solubility is the most difficult problems of these drugs. Drug analysis in final product is important step. There are available some organic solvents such as methanol, chloroform, dimethylformamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out analysis of poorly water-soluble drugs Hydrotropic solubilization is based on analytical method (5). The process is mainly related to quantitative term which is defined as the concentration of the solute in a saturated solution at a certain temperature, and in qualitative terms is defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. The solubility of the drug may be expressed as parts, percentage, molarity, molality, volume fraction and mole fraction.

Table 1: Expression for approximate solubility

Descriptive terms	Relative amounts of solvents to dissolve 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1-10
Soluble	From 10-30
Sparingly soluble	From 30-100
Slightly soluble	From 100-1000
Very soluble	From 1000-10,000
Insoluble or practically insoluble	More than 10,000

Hydrotropy solubilisation is process of addition of a large amount of the second solute results which increase in the aqueous solubility of another solute. There are generally consist of two hydrotropic salts known as, anionic part and hydrotropic aromatic ring. It contains the ionic organic salts, which increase the solubility in the solvent are "salt in" and those salts which decrease solubility are "salt out" solute.

Many solvents have large anion and cation which is completely solubilize in water called Hydrotropic salts.

This phenomenon known as "hydrotropism". It does not show colloidal properties (6).

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Characteristics of Hydrotropes-

- Completely soluble in water and practically insoluble in the system.
- Hydrotropes are surface active and aggregate in aqueous solution because of their amphiphilic structure.
- Should not produce any temperature when dissolved in water.
- Cheap and easy availability.
- Nontoxic and non-reactive.
- Insensitive to temperature effects, when dissolved in water.
- The solvent character being independent of pH, high selectivity, and the absence of emulsification are the other unique advantages of the hydrotrope.

Advantages of Hydrotropic Solubilization

- Hydrotropy is suggested to be superior to other solubilization methods, such immiscibility, micellar solubilization, co-solvency and salting in, because the solvent characteristics are independent of pH, and have high selectivity and does not require emulsification.
- It only requires mixing the drug with the hydrotrope in water.
- It does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion system (7).

- **DRUG PROFILE**

In the proposed study an attempt will be made to develop a HPLC method for simultaneous estimation of Sacubitril and Valsartan (9). This new drug was discovered and developed as tablet dosage form called ENTRESTO by Novartis, which was then approved by US Food and Drug Administration (FDA) in July 2015 for the treatment of heart failure. ENTRESTO is a combined dosage form which contain sacubitril (SAC), a prodrug that results in neprilysin inhibition and

valsartan (VAL) which is angiotensin II Type-1 receptor blocker), is the first medicine in this category. This combined drug (previously known as LCZ696) is also a useful antihypertensive drug (10). Sacubitril or Valsartan is a first-in-class angiotensin receptor- neprilysin inhibitor (ARNi) approved for the treatment of HF.

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- ***Valsartan***

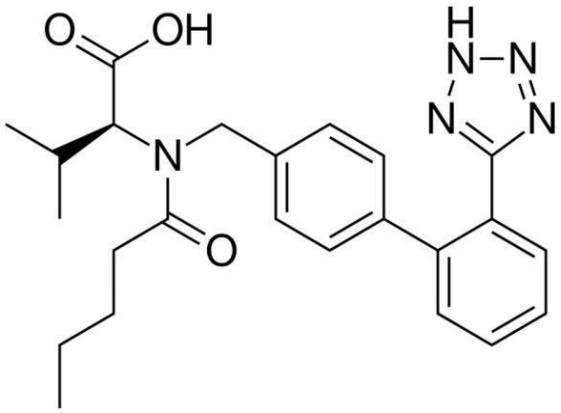

Figure 2: Chemical structure of valsartan

Valsartan is a tetrazole derivative; (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid with molecular formula C24H29N5O3...chemical structure is shown in figure.

History of Valsartan- VAL was first developed by Novartis and was sold under the brand name DIOVAN. It is also available in combination with other antihypertensive drugs.

Physiological properties of valsartan: Valsartan is a white coloured powder that is freely soluble in ethanol, methanol and acetonitrile and sparingly soluble in water. Valsartan appears in the melting range of 105-110˚C and the specific rotation [α] D/20 in methanol being 68˚. The partition coefficient of Valsartan is 0.033 (log P=1.499), suggesting that the compound is hydrophilic at physiological pH. The compound is stable under storage in dry conditions VAL is soluble in the neutral pH range (11).

- ***Sacubitril***

Sacubitril is chemically designated as 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5oxo-2-pentanyl]amino}-4-oxobutanoic acid. Its molecular formula is C24H29NO5, and its molecular weight is 411.49 g/mol (11).

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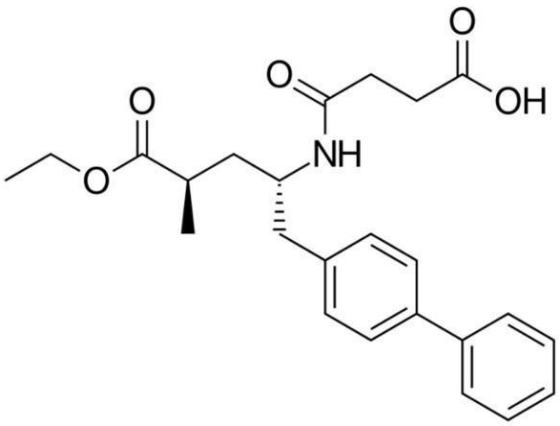

Figure 3: Chemical Structure of Sacubitril

Sacubitril is a neprilysin inhibitor and is used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV). The combination drug, sacubitril/valsartan is used in place of an ACE inhibitor or ARB. It was approved under the FDA‟s priority review process for use in heart failure on July 7, 2015 {8}.

Physical and chemical properties of both drugs Sacubitril/Valsartan complex comprises of anionic forms of sacubitril and valsartan, sodium cations, and water molecules in the molar ratio of 1:1:3:2.5, respectively. A single complex consists of 6 valsartan anions, 6 sacubitril anions, 18 sodium cations, and 15 molecules of water, resulting in the molecular formula C288H330N36Na18O48 _15H2O and a molecular mass of 5748.03 g/ mol.6,13 The substance is a white powder consisting of thin hexagonal plates. It is stable in solid form as well as in aqueous (watery) solution with a pH of 5 to 7, and a melting point of about 138 C (280 F) (12).

The main uses of both drugs are valsartanis used in Hypertensive, diabetic nephopathy and heart failure and sacubitril is used as antihypertensive drug and it is a prodrug which used in combination with valsartan.

MATERIAL & METHOD

The Instrument

The instrument used for all analysis was the ANALYTIK JENA- SPECORD 210 PLUS spectrophotometer recording.

Instpecrumental modes

1)Photometric mode

2)Spectrum mode

3)Quantitation mode

4)Kinetics mode

5)Time scan mode

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6)Multi component
7)Multi wavelength mode
8)Utilities mode

MATERIAL

Selection of Drug

SACUBITRIL and **VALSARTAN** are two drugs which were selected for work after Literature survey the previous study reveals that there are reported work on Sacubitril and Valsartan on UV and HPLC but no work is reported on simultaneous as well as on absorbance method.

Selection of Solvent

Methanol: Water (60:40) was selected as the solvent after considering the solubility and stability factor of both the drugs as well as the interference due to excipient matrix present in the tablet formulation

METHODOLOGY

METHOD – I

SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN TABLET DOSAGE FORM BY USING SIMULTANEOUS EQUATIONS METHOD

Preparation of Standard Stock Solutions of SAC and VAL:

100 mg of Sacubitril (SAC) and 100 mg of Valsartan (VAL) were independently measured and transferred to two 100 ml volumetric bottles. Each drug was dissolved in 60 ml of methanol and shaken by hand for 10 minutes. The volume was formed by marking with water and the final available energy was 1000 µg / ml.

Determination of λ max:

From the stock solutions, .1 ml of SAC and .1 ml of VAL was transferred to two separate 10 ml volumetric flasks and the volume was adjusted to the mark with solvent i.e. strength obtained was 10 µg/ml for SAC and 10 µg/ml for VAL. Both the drug solutions were scanned separately between 200 nm to 400 nm. The overlain spectrum of both drugs was recorded (Shown in **Fig 3**) and two wavelengths 226.0 nm (λ max of SAC) nm and 254.0 nm (λ max of VAL) were selected for estimation of drugs using simultaneous equation method.

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Study of Linearity for SAC:

Appropriate known volumes of aliquots from standard SAC stock solution were transferred to five separate 10 ml volumetric flasks. The volume was adjusted to the mark with Solvent to obtain concentrations of 4, 6, 8, 10, and 12; µg/ml. Absorbance's of these solutions were recorded at 226.0 nm and 254 nm. (Shown in **Table 1 and Table 2**) and Calibration curve was plotted, absorbance *vs* concentration (shown in **Fig. 4 and Fig 5)**.

Table No: 1. Linearity study of SAC at 226.0 nm

Sr.No.	Concentration of SAC in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	4	0.167 ± 0.0015	0.95
2	6	0.252 ± 0.0016	0.65
3	8	0.344 ± 0.0016	0.49
4	10	0.418 ± 0.0017	0.39
5	12	0.510 ± 0.0015	0.30

Table No: 2. Linearity study of SAC at 254.0 nm.

Sr.No.	Concentration of SAC in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	4	0.160 ± 0.0013	0.80
2	6	0.242 ± 0.0011	0.47
3	8	0.324 ± 0.0011	0.35
4	10	0.400 ± 0.0012	0.28
5	12	0.483 ± 0.0013	0.27

Study of Linearity for VAL:

Appropriate known volumes of aliquots from standard VAL stock solution were transferred to five separate 10 ml volumetric flasks. The volume was adjusted to the mark with Solvent to obtain concentrations of 2, 4, 6, 8 and 10 µg/ml. Absorbance of these solutions were measured at 226 nm and 254.0 nm. (**Table 3 an Table 4**) and the calibration curve were plotted, absorbance *Vs* concentration (shown in **Fig. 6 and Fig. 7)**

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Table No: 3. Linearity study of VAL at 226.0 nm.

Sr.No.	Concentration of VAL in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	2	0.092 ± 0.0015	1.71
2	4	0.184 ± 0.0015	0.85
3	6	0.240 ± 0.0015	0.57
4	8	0.363 ± 0.0015	0.43
5	10	0.457 ± 0.0016	0.33

Table No: 4. Linearity study of VAL at 254.0 nm

Sr.No.	Concentration of VAL in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	2	0.224 ± 0.0020	0.92
2	4	0.448 ± 0.0019	0.43
3	6	0.672 ± 0.0020	0.30
4	8	0.889 ± 0.0019	0.21
5	10	1.099 ± 0.0019	0.17

Determination of Absorptivity values of drugs at selected wavelengths:

Absorptivity values of SAC and VAL were calculated by using following formula :- a (1%, 1cm) = A/C
L

Where, a = absorptivity, A = absorbance,

C = concentration gm/100ml,

L = path length

Results of absorptivity values of drugs are shown in Table 5.

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Table No: 5. Absorptivity Values of SAC and VAL at 226.0 nm and 254.0 nm

Sr.No.	Absorptivity at 226.0 nm		Absorptivity at 254.0 nm	
	SAC	**VAL**	**SAC**	**VAL**
1	417.5	460.0	400.0	1120.0
2	420.0	457.5	403.3	1120.0
3	417.5	458.33	405.0	1120.0
4	418.0	456.25	409.0	1111.25
5	425.0	457.0	402.5	1099.0
Mean	**ax1**=419.66	**ax2**=458.31	**ay1** =404.13	**ay2**=1107.40

A set of two simultaneous equations were framed using these absorptivity coefficient values are given below.

A1 = 419.66 * C X + 404.13 *CY ----------**I**

A2 = 458.31 * C X + 1107.40 *CY ----------**II**

Where, **CX** and **CY** are the concentrations of SAC and VAL, respectively in grams per 100 mL in the sample solution.

A1 and **A2** are the absorbance of the sample solution measured at 226.0 nm and 254.0 nm, respectively.

By rearranging equations **I and II.**

Concentrations **CX** and **CY** can be obtained as:

$$CX = \frac{A2 \times 458.31 - A1 \times 1107.40}{-279514.6637} \quad \textbf{III}$$

$$CY = \frac{A1 \times 404.13 - A2 \times 419.66}{-279514.6637} \quad \textbf{IV}$$

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- **Laboratory mixture analysis by proposed method:**

In order to demonstrate the concurrence of the proposed simultaneous evaluation of SAC and VAL in the development of manufactured drugs, this approach was first attempted to balance drugs in a standard laboratory association.

A well-balanced dose of 100 mg SAC and 100 mg VAL was taken in a 100 ml volumetric bottle and dissolved in 60 ml of Methanol by vigorous shaking. The volume is built into the marker with distilled water. The aliquot portions of this stock solution were further diluted with solvent to get final concentration of SAC and VAL in ratio 4:8 and 6:6 ¼g/ml respectively and the absorbance were measured at 226.0 nm and 254.0 nm against solvent as blank.

Amount of each drug was estimated using **III**, **IV** equation as follows and results are reported in **Table 6.**

Table 6: Analysis of SAC and VAL in laboratory mixture:

Sr.No.	Amount Taken mg/mL	Amount found* mg/mL	Amount found (%)	SD	% RSD
1	SAC 4	4.04	101	0.0016	0.30
2	VAL 8	7.96	99.67	0.0008	0.07
3	SAC 6	6.17	102.98	0.0008	0.14
4	VAL 6	6.23	103.88	0.0016	0.17

* No. of three estimations

- **Application of Proposed Method For Analysis of tablet Formulation :**

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed sample equivalent to 100 mg of SAC and 100 mg of VAL was taken in a stoppered volumetric flask (100.0ml); 60ml of Methanol was added and sonicated for 10 min. The solution is filtered through Whatmann's filter paper (No. 41) and the volume is marked with the same water included. The aliquot components of the above solutions were then diluted with a solvent to obtain a final concentration of about 6 (g / ml SAC and 6 (g / ml VAL, respectively and the reductions were measured at 226.0 nm and 254.0 nm in the negative. the drugs in the sample were determined using figures 3 and 4. The results are reported in **Table 7**

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Table 7: Application of Proposed Method for Analysis of Tablets

Sample	Label claimed	% Label claim*	± S.D	%RSD
1	SAC 24mg	97.00	.0016	0.31
	VAL 26mg	102.20	.0024	0.26

*Mean of three observations

- **Validation of Proposed method:**
- The Proposed method was validated as per the ICH guidelines.
- **Accuracy :**
- To assess the accuracy of the improved methods and to study the deformity of structural additives, the analytical recovery tests are performed in a standard add-on. The recovery study was performed by adding 80, 100 and 120% of the test torture according to the ICH guidelines. The known amount of drug added to the preanalyzed tablet powder 6 µg / ml of SAC and 6 µg / ml of VAL and the percentage return is calculated. The results and statistics for SAC and VAL are reported in Table 8.

Table 8: Recovery Study Data

Sr.No.	Concentration of Drug Added (µg/mL)		% Recovery ± SD	
	SAC	VAL	SAC	VAL
1	4.8	4.8	97.22 ± 0.16	101.89 ± 0.16
2	6.0	6.0	97.16 ± 0.08	101.95 ± 0.16
3	7.2	7.2	96.68 ±0.08	102.22 ± 0.08

*Mean of three observations

- **Precision:**
- Precision is determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the 4, 6, 8, ¼g/mL of SAC and 2, 4, 6¼g/mL of VAL of drug solutions for three times in the same day. Inter-day precision was determined by analyzing the 4, 6, 8, ¼g/mL of SAC and 2, 4, 6 ¼g/mL of VAL of drug solutions daily for three days, results are reported in **Table 9.**

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Table 9: Intraday and Interday precision

SR.NO	Drug	Conc.	Intraday*	%RSD	Interday *	%RSD
		4.0µg/ml	4.05	0.48	3.98	0.98
1	**SAC**	6.0µg/ml	5.97	0.65	6.07	0.64
		8.0µg/ml	7.97	0.48	8.04	0.48
		2.0µg/ml	2.01	0.36	1.92	0.74
2	**VAL**	4.0µg/ml	3.98	0.36	4.02	0.36
		6.0µg/ml	6.03	0.24	6.07	0.25

*Mean of three observations

- **Repeatability:**

Repeatability was determined by analyzing SAC (6 ¼g/mL) and VAL (6 ¼g/mL) concentration of drug solutions for five times and results are reported in T**able 10**

Table 10: Repeatability data

Sr.No	Drug	Amount taken* (µg/mL)	Amount found (µg/mL)	%RSD
1	SAC	6	5.97	0.65
2	VAL	6	6.05	0.30

*Mean of five observation

- **Ruggedness:**

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions and the results are reported in **Table 11**

Table 11: Ruggedness Data

Drug	Amount taken (µg/ml)	Analyst I*	%RSD	Analyst II *	%RSD
SAC	6.0	5.97	0.65	6.16	0.61
VAL	6.0	6.05	0.30	6.25	0.23

*Mean of five estimation

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- **Linearity**

For each drug , appriote dilutions of standard stock solution were assayed as per the developed method. The Beer s Lambert s concentration range was found to be 4-20mg/ml for SAC and 2-10mg/ml for VAL at their respective selected wavelengths. Coefficient of correlation was found to be 0.9996 for SAC and 0.9998 for VAL.

METHOD II

SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN TABLET DOSAGE FORM BY USING ABSORBANCE RATIO METHOD

Preparation of Standard Stock Solutions of SAC and VAL:

100 mg of Sacubitril (SAC) and 100 mg of Valsartan (VAL) were measured separately and transferred to two separate volumetric bottles. Each drug was dissolved in 60 ml of methanol and shaken by hand for 10 minutes. The dose was applied to the mark with distilled water and the final strength obtained was 1000 µg / ml.

Determination of » max:

From the stock solutions, .1 ml of SAC and .1 ml of VAL was transferred to two separate 10 ml volumetric flasks and the volume was adjusted to the mark with solvent (methanol: water, 60:40) i.e. strength obtained was 10 µg/ml for SAC and 10 µg/ml for VAL. Both the drug solutions were scanned separately between 200 nm to 400 nm. The overlain spectrum of both drugs was recorded (Shown in **Fig 8**) and two wavelengths 240.0 nm (**isoabsorptive point**) and 254.0 nm (» max of VAL) were selected for estimation of drugs using absorbance ratio method.

Study of Linearity for SAC:

Appropriate known volumes of aliquots from standard SAC stock solution were transferred to five separate volumetric flasks of capacity 10 ml each. The volume was changed to a mark with the solvent Methanol: Water (60:40) to determine the 4, 6, 8, 10, and 12; µg / ml. Absorbance s of these solutions were recorded at 240.0 nm and 254 nm. (**Shown in Tables 12 and Table**

- and the Calibration curve were plotted, absorbance vs concentration (**shown in Fig. 9 and Fig. 10**).

Table No: 12. Linearity study of SAC at 240.0 nm

Sr.No.	Concentration of SAC in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	4	0.160 ± 0.0014	0.88
2	6	0.238 ± 0.0014	0.59
3	8	0.313 ± 0.002	0.63

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4	**10**	**0.396 ± 0.0014**	**0.35**
5	**12**	**0.476 ± 0.0014**	**0.29**

Table No: 13. Linearity study of SAC at 254.0 nm.

Sr.No.	Concentration of SAC in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	**4**	**0.160 ± 0.0013**	**0.80**
2	**6**	**0.242 ± 0.0011**	**0.47**
3	**8**	**0.324 ± 0.0011**	**0.35**
4	**10**	**0.400 ± 0.0012**	**0.28**
5	**12**	**0.483 ± 0.0013**	**0.27**

Study of Linearity for VAL:

Appropriate known volumes of aliquots from standard VAL stock solution were transferred to five separate volumetric flasks of capacity 10 ml each.

The volume was changed to a mark with the solvent Methanol: Water (60:40) to determine the 4, 6, 8, 10, and 12; µg / ml. Absorbance s of these solutions were recorded at 240.0 nm and 254 nm. (**Table 14 and Table 15**) and the calibration curve were plotted, absorbance *Vs* concentration (shown in **Fig. 11 and Fig. 12)**

Table No: 14. Linearity study of VAL at 240.0 nm

Sr.No.	Concentration of VAL in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	**2**	**0.092 ± 0.0014**	**1.72**
2	**4**	**0.163 ± 0.0028**	**1.73**
3	**6**	**0.243 ± 0.0012**	**0.52**
4	**8**	**0.327 ± 0.0010**	**0.33**
5	**10**	**0.408 ± 0.0006**	**0.15**

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Table No: 15. Linearity study of VAL at 254.0 nm.

Sr.No.	Concentration of VAL in (µg/ml)	Absorbance Mean ± SD (n=5)	%RSD
1	2	0.224 ± 0.0020	0.92
2	4	0.448 ± 0.0019	0.43
3	6	0.672 ± 0.0020	0.30
4	8	0.889 ± 0.0019	0.21
5	10	1.099 ± 0.0019	0.17

Determination of Absorptivity values of drugs at selected wavelengths:

Absorptivity values of SAC and VAL were calculated by using following formula :- a (1%, 1cm) = A/C L

Where,

a = absorptivity,

A = absorbance,

C = concentration gm/100ml

, L = path length

Results of absorptivity values of drugs are shown in Table 16.

Table No: 16. Absorptivity Values of SAC and VAL at 240.0 nm and 254.0 nm

Sr.No.	Absorptivity at 240.0 nm		Absorptivity at 254.0 nm	
	SAC	VAL	SAC	VAL
1	395.0	410.0	400.0	1120.0
2	400.0	407.5	403.3	1120.0
3	396.66	405.0	405.0	1120.0
4	391.25	408.75	409.0	1111.25
5	396	408.0	402.5	1099.0
Mean	ax1=395.92	ax2=408.34	ay1 =404.13	ay2=1107.40

The concentration of two drugs in the mixture can be calculated by using equations CSAC = QM

Qy/QX - QY × A1/ax1 ----------**(I)**

CVAL = QM -QX/QY - Qx × A 1 /ax2 ----------**(II)**

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Where, A1 is absorbance of mixture at 240nm (isoabsorptive point) and ax1 (395.92), ax2 (408.34) and ay1 (404.13), ay2 (1107.40) are absorptivity of SAC and VAL at 240nm and 254nm respectively and QM = A2/A1, QY = ay2/ay1 and QX = ax2/ax1.

- **Laboratory mixture analysis by proposed method:**

In order to demonstrate the concurrence of the proposed simultaneous evaluation of SAC and VAL in the development of manufactured drugs, this approach was first attempted to balance drugs in a standard laboratory association.

A well-balanced dose of 100 mg SAC and 100 mg VAL was taken in a 100 ml volumetric bottle and dissolved in 60 ml of Methanol by vigorous shaking. The volume is built into the tag with the same water included. The aliquot portions of this stock solution were further diluted with solvent to get final concentration of SAC and VAL in ratio 4:8 and 6:6 µg/ml respectively and the absorbance were measured at 240.0 nm and 254.0 nm against solvent as blank.

Amount of each drug was estimated using **I**, **II** equation as follows and results are reported in **Table 17.**

Table 17: Analysis of SAC and VAL in laboratory mixture:

Sr.No.	Amount Taken mg/mL	Amount found* mg/mL	Amount found (%)	SD	% RSD
1	SAC 4	3.89	97.25	0.0016	0.33
2	VAL 8	8.02	100.25	0.0008	0.07
3	SAC 6	6.04	100.66	0.0008	0.16
4	VAL 6	6.28	104.66	0.0016	0.17

* No.of three estimations

- **Application of Proposed Method For Analysis of tablet Formulation :**

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed sample equivalent to 100 mg of SAC and 100 mg of VAL was taken in a stoppered volumetric flask (100.0ml); 60ml of Methanol was added and sonicated for 10 min. The solution is filtered through Whatmann's filter paper (No. 41) and the volume is applied to the mark with distilled water. The aliquot components of the above solutions were then diluted with solvent to obtain a final concentration of about 6 (g / ml SAC and 6 (g / ml of VAL, respectively and the reductions were measured at 240.0 nm and 254.0 nm in the negative. the drugs in the sample were determined using statistics I and II.The results are reported in **Table 18**

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Table 18: Application of Proposed Method for Analysis of Tablets

Sample	Label claimed	% Label claim*	± S.D	%RSD
1	SAC 24mg	95.16	.0006	0.17
	VAL 26mg	102.83	.0024	0.26

*Mean of three observations

- **Validation of Proposed method:** The Proposed method was validated as per the ICH guidelines.

- **Accuracy :**

- To assess the accuracy of the improved methods and to study the deformity of structural additives, the analytical recovery tests are performed in a standard add-on. The recovery study was performed by adding 80, 100 and 120% of the test torture according to the ICH guidelines. The known amount of drug added to the preanalyzed tablet powder 6 µg / ml of SAC and 6 µg / ml of VAL and the percentage return is calculated. The results and statistics for SAC and VAL are reported in **Table 19**.

Table 19: Recovery Study Data

Sr.No.	Concentration of Drug Added (µg/mL)		% Recovery ± SD	
	SAC	VAL	SAC	VAL
1	4.8	4.8	100.75 ± 0.16	104.53 ± 0.16
2	6.0	6.0	100.02 ± 0.16	104.66 ± 0.16
3	7.2	7.2	100.68 ± 0.16	104.62 ± 0.16

*Mean of three observations

- **Precision:**

- Precision is determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the 4, 6, 8, ¼g/mL of SAC and 2, 4, 6¼g/mL of VAL of drug solutions for three times in the same day.

Inter-day precision was determined by analyzing the 4, 6, 8, ¼g/mL of SAC and 2, 4, 6 ¼g/mL of VAL of drug solutions daily for three days, results are reported in **Table 20.**

Table 20 : Intraday and Interday precision

SR.NO	Drug	Conc.	Intraday*	%RSD	Interday *	%RSD
		4.0µg/ml	3.97	0.54	4.08	0.87
1	SAC	6.0µg/ml	5.99	0.39	6.02	0.35
		8.0µg/ml	8.05	0.67	7.96	0.64
		2.0µg/ml	2.01	0.36	1.92	0.74
		4.0µg/ml	3.98	0.36	4.02	0.36
2	VAL					
		6.0µg/ml	6.03	0.24	6.07	0.25

*Mean of three observations

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- **Repeatibility:**

Repeatibility was determined by analyzing SAC (6 ¼g/mL) and VAL (6 ¼g/mL) concentration of drug solutions for five times and results are reported in T**able 21**

Table 21: Repeatibility data

Sr.No	Drug	Amount taken* (µg/mL)	Amount found (µg/mL)	%RSD
1	SAC	6	6.06	0.65
2	VAL	6	6.27	0.30

*Mean of five observations

- **Ruggedness:**

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions and the results are reported in **Table 22**

Table 22: Ruggedness Data

Drug	Amount taken (µg/ml)	Analyst I*	%RSD	Analyst II *	%RSD
SAC	6.0	5.97	0.65	6.16	0.61
VAL	6.0	6.05	0.30	6.25	0.23

*Mean of five estimation

- **Linearity**

For each drug , appriote dilutions of standard stock solution were assayed as per the developed method.The Beer s Lambert s concentration range was found to be 4-20mg/ml for SAC and 2-10mg/ml for VAL at their respective selected wavelengths. Coefficient of correlation was found to be 0.9997 for SAC and 0.9998 for VAL.

RESULT & DISCUSSION

METHOD - I

UV-Spectrophotometric Method for Estimation of Sacubitril and Valsartanin Bulk and Tablet Dosage Form by Simultaneous Equation Method.

UV-spectrophotometric method using simultaneous equation was developed. **SAC** showed absorbance maxima at 226 nm and **VAL** at 254.0 nm. Linearity was observed in the concentration rage of 4 - 20µg/ml for SAC and 2 -10µg/ml for VAL. The proposed method was applied for pharmaceutical formulation and % label claim for SAC and VAL was found to be 97.00 and 102.20, respectively. The recovery studies were carried out at 80, 100, 120 % level and % recovery for SAC

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and VAL was found to be 96.68 97.22 and 101.89-102.22, respectively. The low % RSD indicates the method is accurate and precise and can be used for routine pharmaceutical analysis.

Summary of Validation Parameter

Parameters	**SAC**	**VAL**
Recovery (n=3)	96.68 97.22	101.89 102.22
Precision (%RSD)		
Intra-day (n=3)	0.48 - 0.65	0.24 - 0.36
Inter-day (n=3)	0.48 - 0.98	0.25 - 0.74
Repeatibility (n=5)	0.65	0.30
Ruggedness (%RSD)		
Analyst 1	0.65	0.30
Analyst 2	0.61	0.23

METHOD - II

UV-Spectrophotometric Method for Simultaneous Estimation of Sacubitril and Valsartan in Bulk and Tablet Dosage Form by Absorbance ratio method.

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbance selected were 240 nm **(isoabsorptive point)** and 254 nm **(» max of Valsartan)** Linearity was observed in the concentration range of 4 - 20 µg/mland 2 - 10 µg/ml. The proposed method was applied for pharmaceutical formulation; % label claim for **SAC**and**VAL** was found to be **95.16 and 102.83,** respectively. The recovery studies were carried out at 80, 100, 120 % level and % recovery for SAC and VAL was found to be **100.02 - 100.75 and 104.53 104.66,** respectively.

Summary of Validation Parameters

Parameters	**SAC**	**VAL**
Recovery (n=3)	100.02 – 100.75	104.53 – 104.66
Precision (%RSD)		
Intra-day (n=3)	0.39 - 0.67	0.35 - 0.87
Inter-day (n=3)	0.24 - 0.36	0.25 - 0.74
Repeatibility (n=5)	0.65	0.30
Ruggedness (%RSD)		
Analyst 1	0.65	0.30
Analyst 2	0.23	0.61

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CONCLUSIONS

Spectroscopy is one of the most widely used technique to carry out the analysis of pharmaceutical preparation. UV spectroscopy is based on the measurement of spectrum of sample contains atom. A broad range of techniques are available for the analysis of sacubitril and valsartan in pharmaceutical formulation. These techniques are very useful in the structure elucidation of organic molecule.

In the research work done, a successful attempt for simultaneous analysis of SAC and VAL in tablet formulation by following spectrophotometric methods was made by experimentation based on through literature survey. The spectrophotometric methods developed are:

- Simultaneous Equation Method.
- Absorbance Ratio Method.
- Multicomponent Mode Method.

The simplicity, rapidity, reproducibility, and repeatability of the proposed methods completely fulfill the objective of the research work of simultaneous analysis of this drug combination.

METHOD 1

UV-spectrophotometric method using simultaneous equation was developed. SAC showed absorbance maxima at 226 nm and VAL at 254.0 nm. Linearity was observed in the concentration rage of 4 - 20µg/ml for SAC and 2 -10µg/ml for VAL. The proposed method was applied for pharmaceutical formulation and % label claim for SAC and VAL was found to be 97.00 and 102.20, respectively. The recovery studies were carried out at 80, 100, 120 % level and % recovery for SAC and VAL was found to be 96.68 97.22 and 101.89-102.22, respectively. The low % RSD indicates the method is accurate and precise and can be used for routine pharmaceutical analysis.

METHOD 2

UV-spectrophotometric method using simultaneous equation was developed. **SAC** showed absorbance maxima at 226 nm and **VAL** at 254.0 nm. Linearity was observed in the concentration rage of 4 - 20µg/ml for SAC and 2 -10µg/ml for VAL. The proposed method was applied for pharmaceutical formulation and % label claim for SAC and VAL was found to be 97.00 and 102 .20, respectively. The recovery studies were carried out at 80, 100, 120 % level and % recovery for SAC and VAL was found to be 96.68 97.22 and 101.89-102.22, respectively. The low % RSD indicates the method is accurate and precise and can be used for routine pharmaceutical analysis.

ANALYTIK JENA- SPECORD 210 PLUS UV-Visible double beam recording spectrophotometer was employed for analysis. Simultaneous analysis of SAC and VAL was performed in Methanol. Both the drugs followed beers law in the concentration range utilized during analysis.

All the methods were validated as per ICH norms.

It can be said that above three methods can be used for routine laboratory analysis with precision and accuracy.

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A Brief Author Biography

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