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New Method Development and Validation for the Simultaneous Estimation of Sacubitril and Valsartan in Abulk and Pharmaceutical Dosage Forms

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ABSTRACT

A simple and selective LC method is described for the determination of Sacubitril and Valsartan in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumes of water with detection of 241 nm. Linearity was observed in the range 60-140µg /ml for Sacubitril (r^2 =0.997) and 61-155µg /ml for Valsartan (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Buffer, UV spectrophotometer, high performance liquid chromatography, Acetonitrile, Sacubitril, Methanol, Triethayl amine, Valsartan, c_{18} column.

I.INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements.

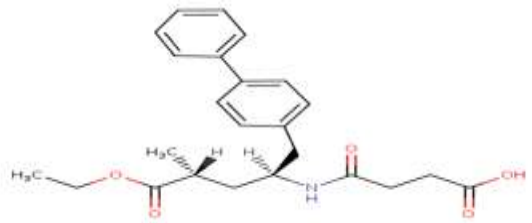
1. Established identity and purity.
2. Established bio availability/dissolution.

II.AIM: To develop new RP HPLC method for the simultaneous estimation of Lamivudine and Tenofovir pharmaceutical dosage form.

PLAN OF WORK: Solubility determination of Lamivudine and tenofovir bromide various solvents and buffers. Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development. Optimize the mobile phase and flow rates for proper resolution and retention times. Validate the developed method as per ICH guidelines.

III. DRUG PROFILE:

3.1. Sacubitril



STRUCTURE:

IUPAC Name	3-{[(2S,4R)-1-([1,1'-BIPHENYL]-4-YL)-5-ETHOXY-4-METHYL-5-OXOPENTAN-2-YL] CARBAMOYL}PROPANOIC ACID
Molecular formula	$C_{24}H_{29}NO_5$
Molecular weight	411.498
Dosage	49mg orally twice a day-initial dose 97 mg orally twice a day-maintenance dose
Uses	To reduce the risk of cardiovascular death and heart failure

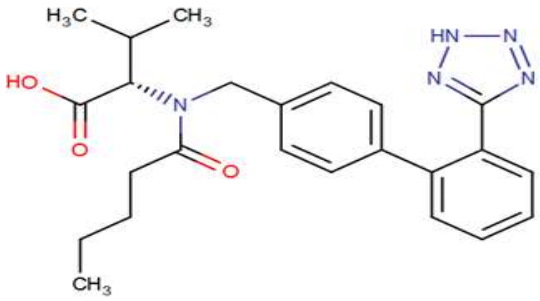
Description: Sacubitril is a neprilysin inhibitor and is used in combination with valsartan to reduce the risk of cardiovascular events in patients with chronic heart failure (NYHA Class II-IV). The combination drug, sacubitril/valsartan is used in place of an ACE inhibitor or ARB. It was approved under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

Mechanism of action:

Sacubitril is a prodrug that is activated to sacubitrilat by de-ehylation 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.

3.2 Valsartan

STRUCTURE:



Description: 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. Valsartan lowers blood pressure by antagonizing the Renin-Angiotensin-Aldosterone System (RAAS); it competes with Angiotensin II for binding to the type-1 Angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of Angiotensin II. Unlike Angiotensin-Converting Enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease**.**

Mechanism of Action: Valsartan is an ARB that selectively inhibits the binding of Angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and Aldosterone-secreting effects of Angiotensin II and results in a decrease in vascular resistance and blood pressure. Valsartan is selective for AT1 and has virtually no affinity for AT2. Inhibition of Aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion. The primary metabolite of valsartan, valeryl 4-hydroxy valsartan, has no pharmacological activity.

Pharmacodynamics:

Valsartan belongs to a class of antihypertensive agents called Angiotensin II receptor blockers (ARBs). Valsartan is a specific and selective type-1 Angiotensin II receptor (AT1) antagonist which blocks the blood pressure increasing effects Angiotensin II via the renin-Angiotensin-Aldosterone system (RAAS). RAAS is a homeostatic mechanism for regulating hemodynamics, water and electrolyte balance. During sympathetic stimulation or when renal blood pressure or blood flow is reduced, renin is released from granular cells of the juxtaglomerular apparatus in the kidneys. Renin cleaves circulating angiotensinogen to Angiotensin I, which is cleaved by Angiotensin converting enzyme (ACE) to Angiotensin II. Angiotensin II increases blood pressure by increasing total peripheral resistance, increasing sodium and water reabsorption in the kidneys via Aldosterone secretion, and altering cardiovascular structure. Angiotensin II binds to two receptors: AT1 and type-2 Angiotensin II receptor (AT2). AT1 is a G-protein

coupled receptor (GPCR) that mediates the vasoconstrictive and Aldosterone-secreting effects of Angiotensin II. Studies performed in recent years suggest that AT2 antagonizes AT1-mediated effects and directly affects long-term blood pressure control by inducing vasorelaxation and increasing urinary sodium excretion. Angiotensin receptor blockers (ARBs) are non-peptide competitive inhibitors of AT1. ARBs block the ability of Angiotensin II to stimulate pressor and cell proliferative effects. Unlike ACE inhibitors, ARBs do not affect bradykinin-induced vasodilatation. The overall effect of ARBs is a decrease in blood pressure.

IV.RESULTS AND DISCUSSION:

4.1. Solubility Studies

These studies are carried out at 25 ^{0}C

Sacubitril:

Sparingly soluble in methanol and in ethanol , very slightly soluble in water and in acetonitrile.

Valsartan:

very slightly soluble in water, soluble in alcohol, freely soluble in chloroform and ether

4.2. Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

4.2.1. Preparation of standard stock solution of Sacubitril:

10 mg of Sacubitril was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

4.2.2. Preparation of standard stock solution of Valsartan:

10 mg of Valsartan was weighed in to 10ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

4.2.3. Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 230 nm for Sacubitril,228 nm for Valsartan and 241 nm for the combination.

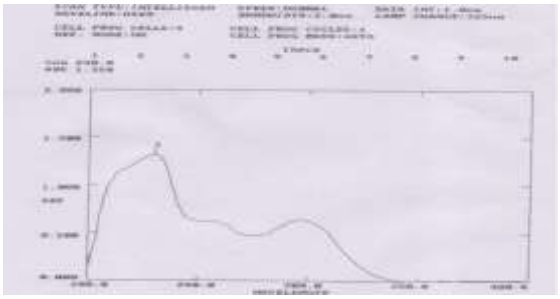


Fig. 4.1: UV-VIS spectrum of Sacubitril,

Observation: λ_{max} was found to be 230 nm for Sacubitril, shown in the figure 4.1

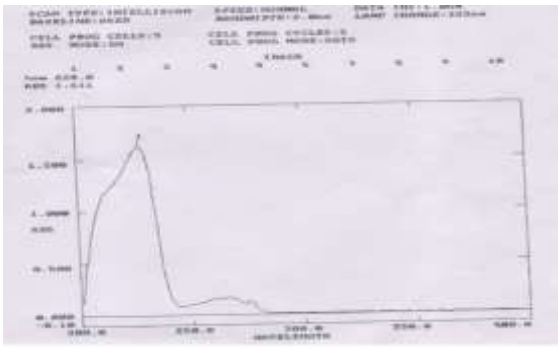


Fig. 4.2: UV-VIS spectrum of Valsartan

Observation: λ_{max} was found to be 228nm for Valsartan shown in the figure 4.2

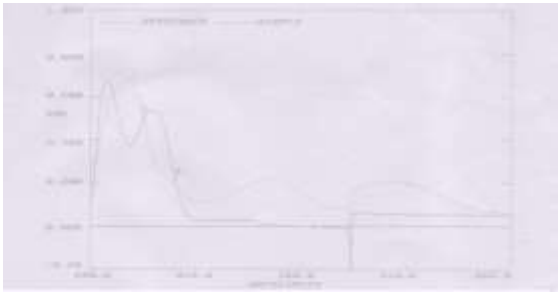


Fig. 4.3: UV-VIS spectrum of sacubitril and valsartan and the isosbestic point was 241nm

Observation: The Isobestic point was found to be 241nm for sacubitril and valsartan in combination and was shown in figure 4.3

4.3. METHOD DEVELOPMENT OF SACUBITRIL AND VALSARTAN

Trial - 1

Chromatographic conditions

Mobile phase : Methanol : ACN : water

Ph : 5.0

Ratio : 50:10:40

Column : Inertsil ODS 3V (250×4.6× 5µ)

wavelength : 241 nm

Flow rate : 1ml/min

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 98 µg/ml of Sacubitriland 102 µg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

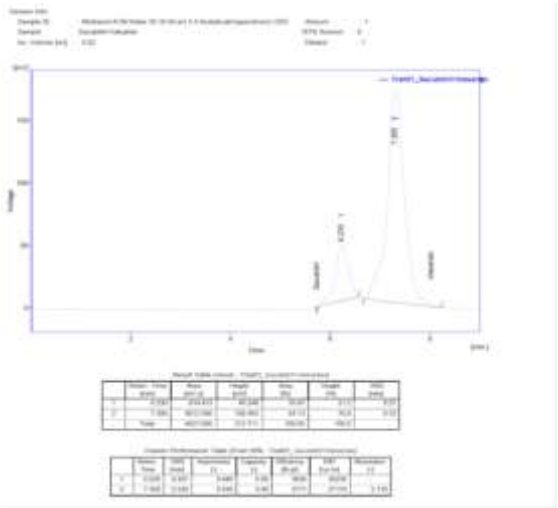


Fig. 4.3.1: Chromatogram of sacubitril and valsartan by using mobile phase

Observation:

- Although the Efficiency was not satisfactory for valsartan.
- The peak response of Sacubitril was very less.
- Hence it was not taken for optimization. The details are given in table 4.3.1 and figure 4.3.1.

Trial- 2

Chromatographic conditions

Mobile phase : Methonol + ACN + Phosphate buffer pH : 4.5

Ratio : 50:30:20

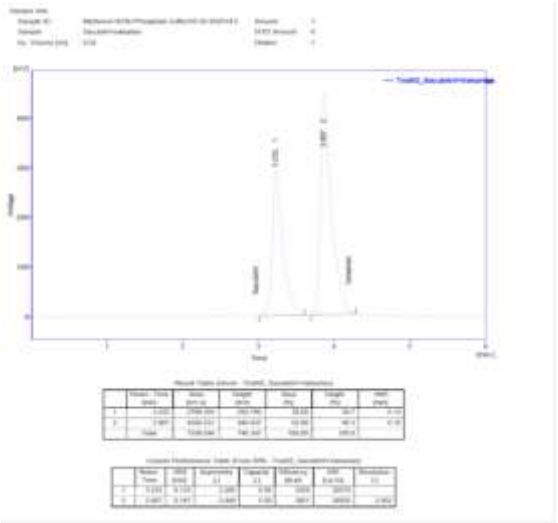
Column : InertsilODS 3V (250×4.6 ×5µ)

wavelength : 241nm

Flow rate : 1ml/min

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 98 µg/ml of Sacubitril and 102 µg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



- **Fig. 4.3.2:** Chromatogram of Valsartan and Sacubitril by using Mobile phase

Observation:

- Efficiency of both the drugs was good.
- The run time is very more.
- The peaks of Sacubitril and Valsartan showed tailing.
- The details are given figure 4.3.2, Hence it was not taken for optimization.

Trial- 3:

Chromatographic conditions

Mobile phase : Phosphate buffer:ACN:Methanol

pH : 4.0

Ratio : 30:30:40

Column : Inertsil ODS 3V, (250×4.6× 5µ)

Wavelength : 241nm

Flow rate : 1ml/min

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 98 µg/ml of Sacubitril and 102 µg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

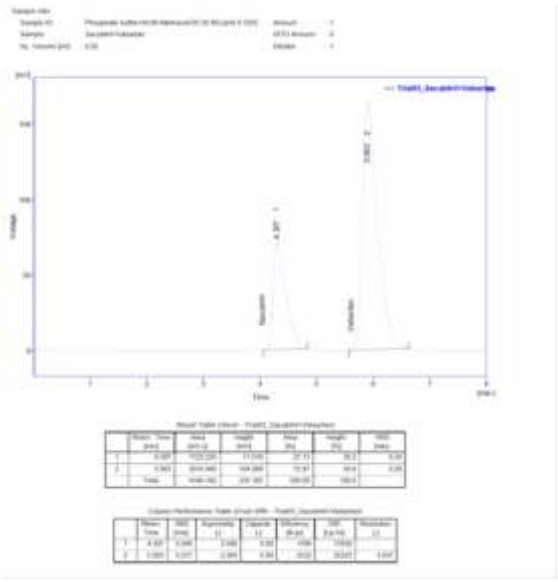


Fig. 4.3.3: Chromatogram of Valsartan and Sacubitril by using mobile phase

Observation:

- Asymmetry factor for VALSARTAN does not meet the system suitability requirements.
- The run time is 11 minutes.
- The details are given figure 6.3.3, hence it was not taken for optimization.

Trial- 4:

Chromatographic conditions

Mobile phase : Mixed phosphate buffer: METHANOL :ACN

pH : 4.5

Ratio : 30:50:20

Column : Inertsil ODS , (250×4.6× 5µ)

Wavelength : 241 nm

Flow rate : 1ml/min

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 98 µg/ml of Sacubitriland 102 µg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

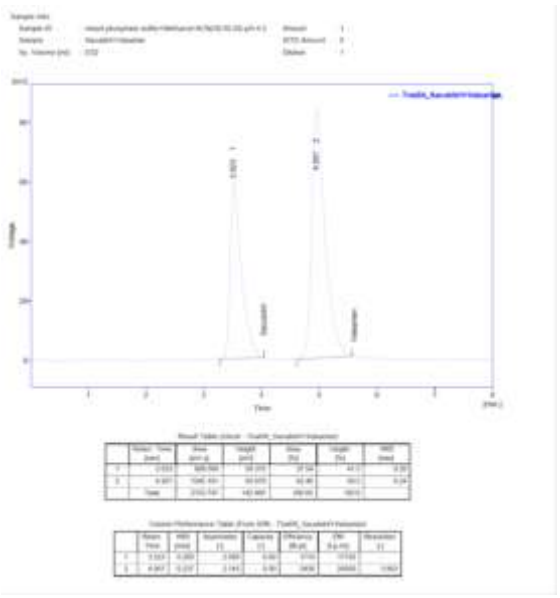


Fig. 4.3.4: Chromatogram of Valsartan and Sacubitril by using mobile phase

Observation:

- Peak Asymmetry factor for Sacubitril and Valsartan does not meet the system suitability requirements.
- The run time is very more.
- The details are given in figure 8.3.4, hence it was not taken for optimization.

Trial-5: (Optimized):

Chromatographic conditions

Mobile phase : Mixed phosphate buffer:MeOH:ACN

pH : 3.0

Ratio : 30:50:20

Column : Inertsil ODS 3V column,C18(250x4.6 ID) 5µm

Wavelength : 241 nm

Flow rate : 1.0ml/min

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 98 µg/ml of Sacubitril and 102µg/ml Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

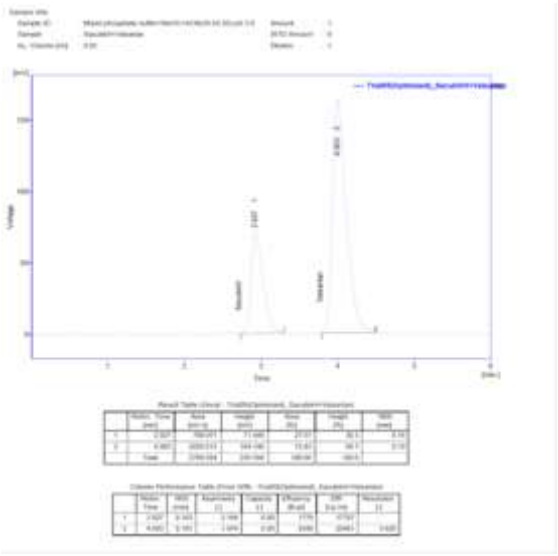


Fig. 4.3.8: Chromatogram of Valsartan and Sacubitril by using mobile phase

Observation:

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both Valsartan and Sacubitril.
- The efficiency was more than 2000 Valsartan and Sacubitril.
- Resolution between two peaks >1.5.
- The details are given in figure 4.3.8, hence this method was for optimized.

Table 4.3.8: Optimized chromatographic conditions

Mobile phase	Mixed buffer: MeOH :ACN (30:50:20)
pH	3.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	241
Injection volume	20 µl
Run time	8 min
Retention time	About 3.420 min for SACUBITRIL and 4.567min for VALSARTAN

4.4. Assay

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 98 mg of Sacubitril and 102 mg of Valsartan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 98 µg/ml of Sacubitril and 102 µg/ml of Valsartan is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tabletsample: 5tablets(each tablet contains Valsartan 102mg

Sacubitril -98 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Valsartan and Sacubitril (µg/ml) were prepared by dissolving weight equivalent to 102 mg of Valsartan and 98 mg of Sacubitril and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 102 µg/ml of Valsartan and 98µg/ml of Sacubitril was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Valsartan and Sacubitril present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Valsartan / Sacubitril in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

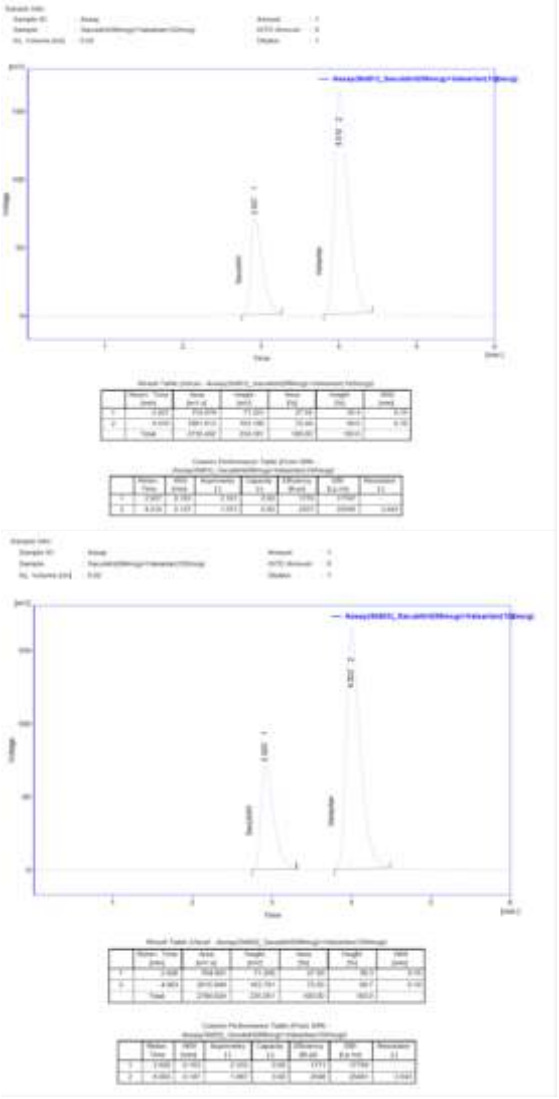


Fig. 4.4.2: Chromatogram of Assay standard preparation-2

Table No.4.4.9.2: Assay Results

	SACUBITRIL		VALSARTAN	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	753.879	766.902	1981.613	2032.155
Injection-2	764.681	772.321	2015.949	2014.800
Injection-3	768.971	762.114	2020.613	2011.585
Injection-4	767.503	763.828	2024.578	2035.808
Injection-5	759.199	755.593	2014.021	2018.668
Average Area	782.848	784.1898	2011.555	2022.439
Standard deviation	6.370728		10.52615	
%RSD	0.008075		0.005205	
Assay(%purity)	100.17		100.55	

Observation

The amount of Sacubitril and Valsartan present in the taken dosage form was found to be 100.17% and 100.55% respectively.

V. CONCLUSION:

"Development and validation of RP-HPLC method for the simultaneous estimation of Sacubitril and Valsartan Pharmaceutical dosage forms". From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Sacubitril and Valsartan was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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