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# DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN DRUG PRODUCT BY RP-HPLC

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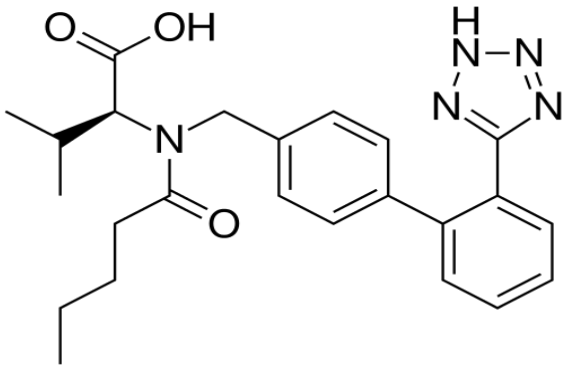
## ABSTRACT
New Analytical method was developed for the estimation of Valsartan and Sacubitril in drug product by liquid chromatography. The chromatographic separation was achieved on C18 column (Xterra RP18 150*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol(25:75). The flow rate was 1.0ml/ minute and ultra violet detector at 267nm. The average retention time for Valsartan and Sacubitil found to be 2.66 min and 3.154 min. the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 51.5 - 154.5µg/ml for Valsartan and 48.5 -145.5µg/ml of Sacubitril.

**KEYWORDS:** Valsartan and Sacubitril, Isocratic, HPLC, Xterra RP18, Formic acid, Acetonitrile, Methanol and validation.

**Valsartan** is an angiotensin II receptor (commonly called angiotensin receptor blocker), Valsartan is mainly used for treatment of high blood pressure, congestive heart failure and to increase the chances of living longer after a heart attack.

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. Since valsartan acts at the receptor, it can provide more complete angiotensin II antagonism since angiotensin II is generated by other enzymes as well as ACE. Also, valsartan does not affect the metabolism of bradykinin like ACE inhibitors.

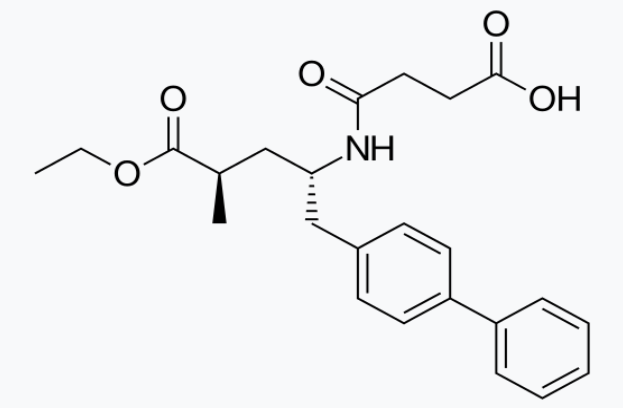
**Valsartan** is chemically designated as (S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido)butanoic acid. Its molecular formula is C24H29N5O3 and its molecular weight is 435.519 g/mol.




**Structure of Valsartan**

**Sacubitril**
Sacubitril is an antihypertensive drug, Sacubitril is a prodrug that is activated to sacubitrilat by de-ethylation via esterase, Sacubitrilat 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 designated as 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid. Its molecular formula is C24H29NO5 and its molecular weight is 411.49 g/mol.




**Structure of Sacubitril**

## EXPERIMENTAL

**Equipments**: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (XTerra RP18 150*4.6,5µ) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

**Materials**: Pharmaceutically pure sample of Valsartan/Sacubitril were obtained as gift samples from Fortune pharma training institute, sri sai nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents pvt ltd. Formic acid (AR grade) was from sd fine chem.

**Chromatographic conditions** The sample separation was achieved on a (5 µ, 150 cm X 4.6 mm i.d.) Xterra Rp18 column, aided by mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (25:75). The flow rate was 1.0 ml/ minute and ultra violet detector at 267nm, that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

**Preparation of mobile phase**

**Buffer Preparation**: Taken accurately 1ml of formic acid in 1000mL of water.

**Mobile phase:** Then added 25 volumes of buffer and 75 volumes of Methanol mixed well and sonicated for 5 min.

**Diluents**: Water: Acetonitrile: 50:50 v\v

**Preparation of standard stock solution:** A 51.5 mg of pure Valsartan and 48.5 mg 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 103µg/ml of Valsartan and 97 µg/ml Sacubitril.

**Preparation of sample solution:** Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 103mg of Valsartan and 97mg 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 diluent 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 103 µg/ml of Valsartan and 97 µg/ml Sacubitril.

## RESULTS AND DISCUSSIONS

**Determination Of Working 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 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using Water as blank. The λmax was found to be 267nm.

After several initial trails with mixtures of methanol, water, ACN and 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). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.

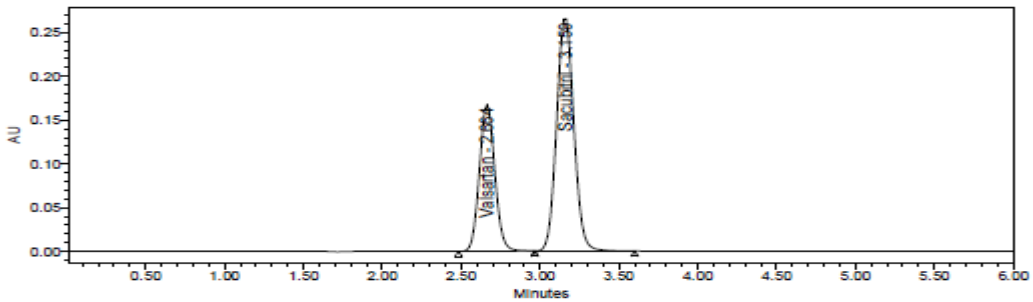



**Figure: 1 Chromatogram of Valsartan/Sacubitril**

## METHOD VALIDATION

### Linearity

Linearity was studied by analyzing five standard solutions covering the range of 51.5 -154.5µg/ml for Valsartan and and 48.5 -145.5µg/ml 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 and 145.5 µg/mL of Sacubitril and 51.5g/mL,77.25µg/mL, 103µg/mL, 128.75µg/mL and 154.5 µg/mL Valsartan.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

**Table No: 1: Linearity data of valsartan**

| Level | Concentration (mg/mL) | Peak area |
|---|---|---|
| 50% | 0.052 | 581656 |
| 75% | 0.077 | 826908 |
| 100% | 0.103 | 1124614 |
| 125% | 0.129 | 1401252 |
| 150% | 0.155 | 1673567 |

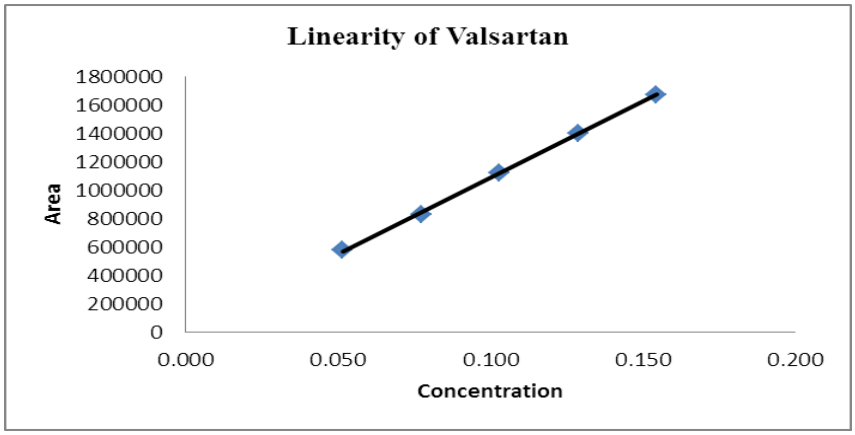



**Figure No.2: Linearity (calibration) curve of Valsartan**

**Table No: 2: Linearity data of Sacubitril**

| Level | Concentration (mg/mL) | Peak area |
|---|---|---|
| 50% | 0.049 | 1055365 |
| 75% | 0.073 | 1489465 |
| 100% | 0.097 | 2005677 |
| 125% | 0.121 | 2473826 |
| 150% | 0.146 | 2923711 |

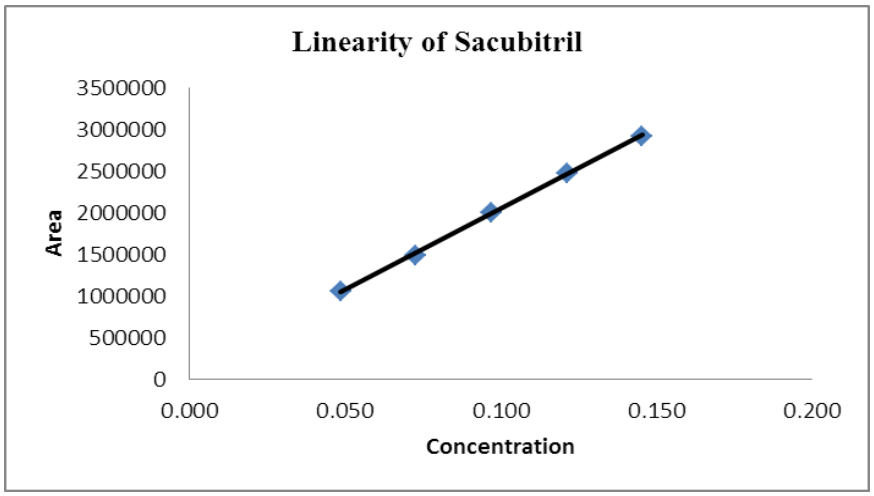



**Figure No.3: Linearity (calibration) curve of Sacubitril**

**RESULT**

A linear relationship between peak areas versus concentrations was observed for Valsartan and Sacubitril in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9997 and 0.9997 for both Valsartan and Sacubitril which prove that the method is linear in the range of 50% to 150%.

**Limit of detection and limit of quantification**

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

**LOD = 3.3 σ /S ……. (1)**

**LOQ =10 σ /S …….. (2)**

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

**Table no. 3: LOD and LOQ values Calculated from calibration curve:**

| | Valsartan mg | Sacubitril mg |
|---|---|---|
| **LOD** | 0.004 | 0.004 |
| **LOQ** | 0.012 | 0.011 |

**Method precision (repeatability)**

The precision of the method was checked by repeated preparation(n=6) of 97µg/ml of Valsartan and 103µg/ml Sacubitril without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times.

***Table.4: Summary of peak areas for method precision of Valsartan***

| Sample No | Retention time | Peak area | % Assay |
|---|---|---|---|
| 1 | 2.665 | 1127298 | 99.8 |
| 2 | 2.665 | 1125239 | 99.6 |
| 3 | 2.665 | 1126274 | 99.3 |
| 4 | 2.664 | 1125675 | 99.2 |
| 5 | 2.665 | 1125561 | 98.4 |
| 6 | 2.666 | 1124930 | 99.4 |
| Mean | 2.665 | 1125830 | 99.3 |
| %RSD | 0.02 | 0.08 | 0.46 |

***Table. 5: Summary of peak areas for method precision of Sacubitril***

| Sample No | Retention time | Peak area | % Assay |
|---|---|---|---|
| 1 | 3.154 | 2009107 | 99.8 |
| 2 | 3.154 | 2001749 | 99.5 |
| 3 | 3.155 | 2007748 | 99.6 |
| 4 | 3.153 | 1998408 | 98.7 |
| 5 | 3.153 | 2006631 | 99.1 |
| 6 | 3.154 | 2004085 | 99.6 |
| Mean | 3.154 | 2004621 | 99.4 |
| %RSD | 0.02 | 0.20 | 0.40 |

**RESULT**

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise.

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating the recoveries of Valsartan and Sacubitril by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Valsartan and Sacubitril. The percentage recovery results obtained are listed in Table 6 & 7.

**Table No. 6: Recovery data of Valsartan**

| LEVEL | S.No | %Recovery of Valsartan | Average |
|---|---|---|---|
| **50** | 1 | 99.7 | 99.4% |
| | 2 | 99.2 | |
| | 3 | 99.3 | |
| **100** | 1 | 99.8 | 99.40% |
| | 2 | 99.6 | |
| | 3 | 99.3 | |
| **150** | 1 | 99.3 | 99.1% |
| | 2 | 98.8 | |
| | 3 | 99.1 | |

**Table No.7: Recovery data of Sacubitril**

| LEVEL | S.No | %Recovery of Sacubitril | Average |
|---|---|---|---|
| **50** | 1 | 99.4 | 99.8% |
| | 2 | 99.7 | |
| | 3 | 100.4 | |
| **100** | 1 | 99.8 | 99.6% |
| | 2 | 99.5 | |
| | 3 | 99.6 | |
| **150** | 1 | 99.9 | 99.2% |
| | 2 | 99.1 | |
| | 3 | 98.7 | |

**RESULT**

Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate.

**Robustness**: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no. 8 & 9).

**Table No. 8: Results of of Valsartan**

| parameter | Rt of Valsartan | Theoretical plates | Asymmetry |
|---|---|---|---|
| Decreased flow rate (0.8ml/min) | 2.229 | 4191 | 1.09 |
| Increased flow rate (1.2ml/min) | 3.316 | 3123 | 1.05 |
| Wave Length 265nm | 2.664 | 3598 | 1.07 |
| 269 | 2.665 | 3627 | 1.07 |

**Table No. 9: Results of of Sacubitril**

| parameter | Rt of Sacubitril | Theoretical plates | Asymmetry |
|---|---|---|---|
| Decreased flow rate (0.8ml/min) | 2.635 | 4820 | 1.08 |
| Increased flow rate (1.2ml/min) | 3.930 | 3620 | 1.06 |
| Wave Length 265nm | 3.159 | 4098 | 1.07 |
| 269 | 3.158 | 4125 | 1.07 |

**RESULT**

The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

**Ruggedness:** The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table no.10&11.

**Table No. 10: Results of Valsartan**

| | | %Assay | %RSD |
|---|---|---|---|
| **Analyst-1** | **VALSARTAN** | 99.8 | 0.14% |
| **Analyst-2** | | 99.6 | |

**Table No. 11: Results of Sacubitril**

| | | %Assay | %RSD |
|---|---|---|---|
| **Analyst-1** | **SACUBITRIL** | 99.8 | 0.21% |
| **Analyst-2** | | 99.5 | |

**RESULT**

The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

**Table No. 12: Summary of Valsartan**

| S.NO | PARAMETER | RESULT | ACCEPTENCE CRITERIA |
|---|---|---|---|
| 1 | System suitability<br>Theoretical plates<br>Asymmetry<br>Retention time<br>%RSD | <br>3583<br>1.07<br>2.663<br>0.22 | <br>Not less than 2000<br>Not more than 1.5<br><br>Not more than 2.0 |
| 2 | Specificity | Specific | Specific |
| 3 | Method precision(%RSD) | 0.46 | Not more than 2.0% |
| 4 | Linearity Range(mcg/ml)<br>Correlation coefficient($r^2$) | 51.5-154.5<br>0.9997 | <br>Not less than 0.990 |
| 5 | Accuracy<br>(Mean % recovery)<br>50%<br>100%<br>150% | <br><br>99.4<br>99.40<br>99.1 | <br><br>97 - 103% |
| 6 | Robustness | All the system suitability parameters are within the limits. | |

*RSD = Relative standard deviation.

**Table No. 13: Summary of Sacubitril**

| S.NO | PARAMETER | RESULT | ACCEPTENCE CRITERIA |
|---|---|---|---|
| 1 | System suitability<br>Theoretical plates<br>Asymmetry<br>Retention time<br>%RSD | <br>4088<br>1.07<br>3.156<br>0.28 | <br>Not less than 2000<br>Not more than 1.5<br><br>Not more than 2.0 |
| 2 | Specificity | Specific | Specific |
| 3 | Method precision(%RSD) | 0.40 | Not more than 2.0% |
| 4 | Linearity<br>Range(mcg/ml)<br>Correlation coefficient($r^2$) | <br>48.5-145.5<br>0.9997 | <br>Not less than 0.990 |
| 5 | Accuracy<br>(Mean % recovery)<br>50%<br>100%<br>150% | <br><br>99.8<br>99.6<br>99.2 | <br><br>97 - 103% |
| 6 | Robustness | All the system suitability parameters are within the limits. | |

## CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of VALSARTAN and SACUBITRIL 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 industries, approved testing laboratories.

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