Analytical Method Development for Sacubitril and Valsartan in Combined Pharmaceutical Dosage Forms by RP-HPLC

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Abstract

The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation of Valsartan and Sacubitril pure drug form. Chromatographic separation was done using Terrosil C18 column having dimension of (100 mm x 4.6 mm) having particle size of 5.0 µm, with mobile phase consisting of Phosphate buffer (KH2PO4 and K2HPO4) pH 3 ±0.02 pH adjusted with ortho phosphoric acid and Acetonitrile (25:75 %v/v), flow rate was adjusted to 0.8 ml/min and detection wavelength at 254nm. The retention times of Valsartan and Sacubitril was found to be 2.589 and 3.711mins. The no. of theoretical plates is 4456, 582 and the tailing factor is 1.4, 1.3.

Keywords: Valsartan, Sacubitril, RP-HPLC, Method development.

Introduction

High Performance Liquid Chromatography (HPLC) [1-2] is most widely used analytical technique. Chromatography process can be defined as separation techniques involving mass-transfer between stationary and mobile phases.

Sacubitril It is an antihypertensive drug used in combination with valsartan. The combination drug valsartan/sacubitril [3], known during trails as LCZ696 and marketed under the brand name Entresto, is a treatment for heart failure it was approved under the FDA’s priorityReview process for use in heart failure on July, 7, 2015.

Chemical Structure

![Chemical Structure Image]

IUPAC Name: 4-[[2S, 4R]-1-(4-Biphenyl)-5-ethoxy-4-methyl-5-oxo-2- pentany] amino]-4-oxobutanoic acid

Chemical formula: C24H29NO5

Molecular weight: 411.49 g/mol
Melting point: 138
Description: white powder
Solubility: Soluble in water, ethanol and methanol
pKa: 3.5
Category: Antihypertensive
**Valsartan**: It is a combination drug for use in heart failure developed by NOVARTIS. It consists of the angiotensin receptor blocker valsartan and the neprilysin inhibitor sacubitril, in a 1:1 mixture by molecule count. It may be used instead of an ACE inhibitor or an angiotensin receptor blocker in people with heart failure with reduced ejection fraction.

**Chemical Structure**

![Chemical Structure](image)

**IUPAC NAME**: (2S)-3-methyl-2-[pentanoyl-([4-[2-(2H-tetrazol-5-yl)]phenyl]phenyl)methyl]amino]butanoic acid

**Chemical formula**: $\text{C}_{24}\text{H}_{29}\text{N}_{5}\text{O}_{3}$

**Molecular weight**: 435.51876 g/mol

**Melting point**: 116-117 °C

**Description**: White, odorless crystalline powder

**Solubility**: Soluble in water, soluble in alcohol

**PKa**: 4.37

**Category**: Anti-hypertensive

**Materials and Methods**

**Experimental Work**

**Table 1: List of instruments used**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Instrument name</th>
<th>Model number</th>
<th>Soft ware</th>
<th>Manufacturers Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HPLC-auto sampler – UV detector</td>
<td>Separation module2695, UV.detector2487</td>
<td>Empower-software version-2</td>
<td>Waters</td>
</tr>
<tr>
<td>2</td>
<td>U.V double beam spectrometer</td>
<td>UV 3000+</td>
<td>U.V win soft ware</td>
<td>Lab India</td>
</tr>
<tr>
<td>3</td>
<td>Digital weighing balance(sensitivity 5mg)</td>
<td>ER 200A</td>
<td>-</td>
<td>Acoset</td>
</tr>
<tr>
<td>4</td>
<td>pH meter</td>
<td>AD 102U</td>
<td>-</td>
<td>ADWA</td>
</tr>
<tr>
<td>5</td>
<td>Sonicator</td>
<td>SE60US</td>
<td>-</td>
<td>Enertech</td>
</tr>
</tbody>
</table>
Table 2: List of chemicals and standards used

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemicals</th>
<th>Manufacturer Name</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>Merck</td>
<td>HPLC grade</td>
</tr>
<tr>
<td>2.</td>
<td>Methanol</td>
<td>Merck</td>
<td>HPLC grade</td>
</tr>
<tr>
<td>3.</td>
<td>Acetonitrile</td>
<td>Merck</td>
<td>HPLC grade</td>
</tr>
<tr>
<td>4.</td>
<td>Ortho phosphoric acid</td>
<td>Merck</td>
<td>G.R</td>
</tr>
<tr>
<td>5.</td>
<td>KH₂PO₄</td>
<td>Merck</td>
<td>G.R</td>
</tr>
</tbody>
</table>

Table 3: Active pharmaceutical Ingredient (pure drug)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan and Sacubitril</td>
<td>Reference Standard</td>
</tr>
</tbody>
</table>

Table 4: Marketed Formulation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>KP LABS</td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>KP LABS</td>
</tr>
</tbody>
</table>

Solubility of drug was observed by dissolving it in different solvents and it was found that drug having good solubility in followings.

Table 5: Solubility of drugs in different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility Valsartan and Sacubitril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
</tr>
</tbody>
</table>

Method Development

Selection of Mobile Phase
The method development [5-9] and validation of Valsartan and Sacubitril requires greater resolution. Hence different solvent systems were tried. The trials are using UV 3000+ equipment with PDA detector and isocratic pump. The system controlled by LC solution software.

Selection of Flow Rate
The flow rate of Valsartan and Sacubitril were tried from 0.8 ml to 1.5ml.

Trial-1
Buffer Preparation
About 7.0g of potassium di hydrogen ortho phosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with ortho phosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of Mobile Phase
Mobile phase consist of water: methanol HPLC of pH 2.5 (30:70) was taken sonicated and degassed for 10 min and filtered through 0.45 µm nylon membrane filter.

Standard Preparation
Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flaskand then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark.
with diluents. The chromatogram was shown in Figure-1

**Chromatographic Conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>ChromosilColumnC&lt;sub&gt;18&lt;/sub&gt; (150mm x 4.6mm)5µg.</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Water: Methanol pH 2.5 (30:70v/v)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8ml/ min</td>
</tr>
<tr>
<td>Detector wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Auto injector (vial)</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20µl</td>
</tr>
</tbody>
</table>

**Table 6: Details of Trail-1**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>4.335</td>
<td>53644</td>
<td>23306</td>
<td>1894</td>
<td>1.4</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>8.844</td>
<td>34197</td>
<td>10561</td>
<td>4153</td>
<td>2.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**Observation**

The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

**Trial-2**

**Buffer Preparation**

About 7.0g of potassium di-hydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

**Preparation of Mobile Phase**

Mobile phase consist of water: acetonitrile of pH 2.5 (30:70) was taken sonicated and degassed for 10 min and filtered through 0.45µm nylon membrane filter.

**Standard Preparation**

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The chromatogram was shown in **Figure-2.**
Column: Chromosil C<sub>18</sub>Column (150mm x 4.6mm)5µg.
Mobile phase: water: Acetonitrile P<sub>H</sub> 2.5 (30:70v/v)
Flow rate: 1ml/ min
Detector wavelength: 254 nm
Injection mode: Auto injector (vial)
Injection: 20µl

![Chromatogram of Valsartan](image)

**Table 7: Details of Trial-2**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>4.333</td>
<td>518384</td>
<td>22211</td>
<td>92</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>6.669</td>
<td>345778</td>
<td>13704</td>
<td>3508</td>
<td>2.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Observation**

The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

**Trial-3**

**Buffer Preparation**

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and P<sub>H</sub> 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

**Preparation of Mobile Phase**

Mobile phase consist of buffer: Methanol of P<sub>H</sub>2.5 (20:80) was taken sonicated and degassed for 10min and filtered through 0.45µm nylon membrane filter.

**Standard Preparation**

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

The chromatogram was shown in **Figure-3.**
Chromatographic Conditions

Column : Xterra C18Column (150mm x 4.6mm) 5µg.
Mobile phase : Phosphate buffer: Methanol PH 2.5 (45:55v/v)
Flow rate : 1ml/ min
Detector wavelength : 254 nm
Injection mode : Auto injector (vial)
Injection volume : 20µl

Fig3: Chromatogram of Valsartan and Sacubitril

Table 8: Details of Trail-3

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>3.231</td>
<td>432752</td>
<td>25062</td>
<td>1439</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>4.900</td>
<td>254934</td>
<td>15048</td>
<td>3584</td>
<td>2.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Observation

The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-4

Buffer Preparation

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and PH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of Mobile Phase

Mobile phase consist of buffer: Methanol of PH2.5 (30:70) was taken sonicated and degassed for 10min and filtered through 0.45µm nylon membrane filter.

Standard Preparation

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phaseup to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.
Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Chromatographic Conditions**

- **Column**: Xterra C18 Column (150mm x 4.6mm) 5µm.
- **Mobile phase**: Phosphate buffer: Methanol pH 2.5 (30:70v/v).
- **Flow rate**: 1ml/ min
- **Detector wavelength**: 254 nm
- **Injection mode**: Auto injector (vial)
- **Injection volume**: 20µl

The chromatogram was shown in Figure-4.

### Table 9: Details of Trail-5

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>3.218</td>
<td>400986</td>
<td>39855</td>
<td>2602</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>5.010</td>
<td>248371</td>
<td>20892</td>
<td>4499</td>
<td>1.6</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**Observation**

The separation of two analytical peaks occurred but fronting occurs in Valsartan peak.

**Trial-5**

**Buffer Preparation**

About 7.0g of potassium di hydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

**Preparation of Mobile Phase**

Mobile phase consist of buffer: Methanol of pH 2.5 (30:70) was taken sonicated and degassed for 10min and filtered through 0.45µm nylon membrane filter.

**Standard Preparation**

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.
Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

**Chromatographic Conditions**

- **Column**: ThermosilC18Column (100mm x 4.6mm) 5µg.
- **Mobile phase**: Phosphate buffer: Methanol pH 2.5 (30:70v/v)
- **Flow rate**: 1ml/ min
- **Detector wavelength**: 254 nm
- **Injection mode**: Auto injector (vial)
- **Injection volume**: 20µl

The chromatogram was shown in **Figure-5**.

![Fig 5: Chromatogram of Valsartan and Sacubitril](image)

**Table10: Details of Trails-5**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>2.593</td>
<td>239603</td>
<td>44771</td>
<td>5354</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>3.715</td>
<td>200189</td>
<td>31439</td>
<td>8104</td>
<td>1.2</td>
<td>7.1</td>
</tr>
</tbody>
</table>

**Observation**

The separation of two analytical peaks was good but base line noise is occurred. So the mobile phase ratio has been changed for next trial.

**Trial-6**

**Buffer Preparation**

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

**Preparation of Mobile Phase**

Mobile phase consist of buffer: Methanol of pH2.5 (35:65) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter.

**Standard Preparation**

Weigh accurately 10mg Valsartan Working Reference Standard and 15mg of Sacubitril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the
above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The chromatogram was shown in Figure-6.

**Chromatographic Conditions**

- **Column**: ThermosilC_{18} Column (100 mm x 4.6 mm) 5µg.
- **Mobile phase**: Phosphate buffer: Methanol pH 2.5 (35:65 v/v)
- **Flow rate**: 1ml/ min
- **Detector wavelength**: 254 nm
- **Injection mode**: Auto injector (vial)
- **Injection volume**: 20µl

![Chromatogram of Valsartan and Sacubitril](image)

**Table 11: Details of Trail-6**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>R(_t)</th>
<th>Area</th>
<th>Height</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valsartan</td>
<td>2.605</td>
<td>2233704</td>
<td>365596</td>
<td>4456</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sacubitril</td>
<td>3.781</td>
<td>1328106</td>
<td>174637</td>
<td>5823</td>
<td>1.3</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**Observation**

The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method

**Results & Discussion**

**Method Development Results**

**Trial-1**

In Trial-1, Mobile phase was Methanol: water (70:30 v/v) and adjust the pH2.5 with ortho phosphoric acid. The Column was Chromosil (150mm x 4.6 mm)5µg. The flow rate was set to 0.8ml/min. Injection volume is 20µl. In this chromatographic condition the separation of two analytical peaks was not proper, So the mobile phase composition was changed for next trail.

**Trial-2**

In Trial-2, Mobile phase was water: Acetonitrile (30:70 v/v) and adjust the pH2.5 with ortho phosphoric acid. The Column was Chromosil (150mm x 4.6 mm)5µg. The flow rate was set to 1ml/min. Injection volume is 20µl. In this chromatographic...
condition both Valsartan and Sacubitril peaks were observed but resolution was not properly. So the mobile phase composition was changed for next trail.

**Trial-3**

In Trial-3, Mobile phase was phosphate Buffer: Methanol (20:80 v/v) and adjust the pH 2.5 with ortho phosphoric acid. The Column was Xterra C18 (150 mm x 4.6 mm) 5µg. The flow rate was set to 1ml/min. Injection volume is 20µl. In this chromatographic condition both Valsartan and sacubitril peaks were observed but resolution was not properly. So the mobile phase composition was changed for next trail.

**Trial-4**

In Trial-4, Mobile phase was phosphate Buffer: Methanol (30:70 v/v) and adjust the pH 2.5 with ortho phosphoric acid. The Column was Xterra C18 (150 mm x 4.6 mm) 5µg. The flow rate was set to 1ml/min. Injection volume is 20µl. In this chromatographic condition both Valsartan and sacubitril peaks were observed but fronting occurs in Valsartan peak. So the mobile phase composition was changed for next trail.

**Trial-5**

In Trial-5, Mobile phase was phosphate Buffer: Methanol (30:70 v/v) and adjust the pH 2.5 with ortho phosphoric acid. The Column was ThermosilC18 (100 mm x 4.6 mm) 5µg. The flow rate was set to 1ml/min. Injection volume is 20µl. In this chromatographic condition both Valsartan and Sacubitril peaks were observed. Separation is good but baseline noise is occurred. So the mobile phase composition was changed for next trail.

**Trial-6**

In Trial-6, Mobile phase was phosphate Buffer: Methanol (35:65 v/v) and adjust the pH 2.5 with ortho phosphoric acid. The Column was ThermosilC18 (100 mm x 4.6 mm) 5µg. The flow rate was set to 1ml/min. Injection volume is 20µl. In this chromatographic condition both Valsartan and Sacubitril peaks were observed. The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method [10].

**Conclusion**

Trial 6 was optimized for the method development of deliberately changing the chromatographic conditions. Column used was Trerosil C18 (100 mm x 4.6 mm) 5µg. Mobile phase composition of buffer: Methanol in the ratio (35:65 V/V) and buffer pH 2.5 adjusted with ortho phosphoric acid. The Flow rate set to 0.8ml min⁻¹ with UV detection was carried out at 254 nm. The results of the present study indicated that the developed method is simple, precise and cost effective for the simultaneous estimation of Valsartan and Sacubitril for routine quality control analysis of these either in bulk and pharmaceutical formulation.

The developed and validated RP-HPLC method outlined is very obvious, affordable, dynamic, low cost, rapid and easy to perform with small sample volume and good repeatability. It can be adopted for the routine quality control analysis of simultaneous determination of Valsartan and Sacubitril because of good resolution of the chromatographic peaks.

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**References**


