Analytical Method Development for Simultaneous Estimation of Cobicistat and Darunavir by RP-HPLC Method

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Abstract

A new method was established for simultaneous estimation of Cobicistat and Darunavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Cobicistat and Darunavir by using Xterra C18 5μm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. Five trails have been done with various composition of mobile phase. Among them the mobile phase composition Acetonitrile and phosphate buffer in the ratio of 70:30 (trail no 5), using C18 column as stationary phase and detection at 255nm has produced a well resoluted peak. The no of theoretical plates was 5105, 3788 with a tailing factor of 1.3, 1.4. The peak retention time was 2.399, 3.907 with a peak area of 946124, 11541. The results shown that developed method is rapid, specific for the simultaneous estimation of cobicistat and darunavir in combined dosage form.

Keywords: Cobicistat, Darunavir, RP-HPLC, Phosphate buffer, ACN

Introduction

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity [1, 2].

RP-HPLC: in RP-HPLC the stationary phase is non-polar in nature and the mobile phase is polar in nature. In this technique polar compounds travel faster and are eluted first. This is because less affinity between solute and stationary phase. non-polar compounds are retained for longer time in the column because more affinity towards stationary phase and takes more time to be eluted from the column.

Darunavir and cobicistat combination is used together with other medicines to treat human immunodeficiency syndrome (AIDS).

Darunavir and cobicistat combination will not cure or prevent HIV infection or AIDS. It helps keep HIV from reproducing and appears to slow down the destruction of immune system. This may help delay problems that are usually related to AIDS or HIV disease from occurring. This medicine will not keep you from spreading HIV to other people.

Cobicistat
Chemical Data


Chemical formula: C_{40}H_{53}N_{7}O_{5}S_{2}

Molecular weight: 776.023 g/mol

CAS No: 1004316-88-4

Mechanism of Action: Cobicistat is a licensed drug for use in the treatment of infection with the human immunodeficiency virus (HIV). cobicistat is of interest for its ability to inhibit liver enzymes that metabolize other medications used to treat HIV, notably elvitegravir, an HIV integrase inhibitor. By combining cobicistat with elvitegravir, higher concentrations of the latter are achieved in the body with lower dosing, theoretically enhancing elvitegravir viral suppression while diminishing its adverse side-effects. Cobicistat is a potent inhibitor of cytochrome P450 3A enzymes, including the important CYP3A4 subtype. It also inhibits intestinal transport proteins, increasing the overall absorption of several HIV medications, including gatazanavir, darunavir.

Solubility: soluble in DMSO (100 mg/mL (128.86 mM)), Soluble in water (<1 mg/mL (<1 mM)), ethanol (100 mg/mL (128.86 mM))

Category: Antiviral agents, cytochrome P450 3A enzyme inhibitor.

Darunavir:

IUPAC Name: [(1S,2R)-3-[[4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamicacid(3R,3aS,6aR)hexahydrofuro[2,3-b]furan-3-ylester monoethanolate

Chemical formula: C_{27}H_{37}N_{3}O_{7}S• C_{2}H_{5}OH

Molecular weight: 593.73

CAS No: 206361-99-1

Physical Data

Mechanism of action: Darunavir (brand name Prezista, formerly known as TMC114) is a protease inhibitor drug used to treat HIV infection. Prezista is an OARAC recommended treatment option for treatment-naïve and treatment-experienced adults and adolescents. Developed by pharmaceutical company Tibotec

Solubility: soluble in water approximately 0.15 mg/mL in at 20°C.

Category: Darunavir is an HIV protease inhibitor. It works by blocking the growth of HIV.
Experimental Work

The list of instruments used in the course of experimental work is as follows:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Instrument</th>
<th>Model No.</th>
<th>Software</th>
<th>Manufacturer's name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HPLC Alliance PDA Detector</td>
<td>Waters 2695 Waters 996</td>
<td>Empower</td>
<td>Waters</td>
</tr>
<tr>
<td>2</td>
<td>UV double beam spectrophotometer</td>
<td>UV 3000</td>
<td>UV Win 5</td>
<td>Lab India</td>
</tr>
<tr>
<td>3</td>
<td>Digital Weighing balance</td>
<td>BSA224SW</td>
<td>-</td>
<td>Satorius</td>
</tr>
<tr>
<td>4</td>
<td>pH meter</td>
<td>AD102U</td>
<td>-</td>
<td>Lab India</td>
</tr>
<tr>
<td>5</td>
<td>Ultra sonicator</td>
<td>SE60US</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Suction pump</td>
<td>VE115N</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The experimental work involves several chemicals. Chemicals used presently are listed below

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chemical</th>
<th>Manufacturer</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>Potassium dihydrogen orthophosphate</td>
<td>Merck</td>
<td>A.R</td>
</tr>
<tr>
<td>5</td>
<td>Cobicistat and Darunavir</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Method Development

Method development for simultaneous estimation of Cobicistat and darunavir in Pharmaceutical dosage forms includes the following steps:

- Selection of detection wavelength (\(\lambda_{\text{max}}\))
- Selection of column
- Selection of mobile phase
- Selection of flow rate
- Preparations and procedures.

**Selection of Detection Wavelength:** 10 mg of Cobicistat and darunavir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Cobicistat and darunavir. The isobestic point was taken as detection wavelength [3].

**Selection of Column:** Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5\(\mu\)m, Make: Waters)]

**Selection of Mobile Phase:** Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved [4].
Selection of Flow Rate

Flow rate is selected based on

- Retention time
- Column back pressure
- Peak symmetry
- Separation of impurities

Preparations and Procedures

Preparation of Phosphate buffer :( PH: 4.6):

Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with Orth phosphoric acid [5,6,7].

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 μ filter under vacuum filtration.

Diluant Preparation:

Mobile phase is used as Diluant.

Preparation of the individual Cobicistat standard preparation:

10 mg of Cobicistat working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluant [8,9].

Preparation of the individual Darunavir standard preparation:

10mg of Darunavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluents [10-14].

Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Darunavir and Cobicistat (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted up to the mark with diluant

Procedure:

20μL of the standard, sample are injected into the chromatographic system and the areas for Darunavir and Cobicistat peaks are measured and the % Assay are calculated by using the formulae.
**System Suitability:**

Tailing factor for the peaks due to Darunavir and Cobicistat in Standard solution should not be more than 2.0. Theoretical plates for the Darunavir and Cobicistat peaks in Standard solution should not be less than 2000.

**Assay calculation:**

\[
\text{Assay \%} = \left( \frac{\text{sample area}}{\text{Standard area}} \right) \times 100 \times \left( \frac{\text{dilution sample}}{\text{dilution of standard}} \right) \times \left( \frac{P}{100} \right) \times \left( \frac{\text{Avg. wt}}{Lc} \right)
\]

Where,

\[
P \quad \text{Percentage purity of working standard}
\]

\[
Lc \quad \text{LABEL CLAIM OF drug in mg/ml}
\]

**Results and Discussion**

**Method Development:** The chromatographic method development for the simultaneous estimation of Cobicistat and Darunavir were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Cobicistat and Darunavir in API and pharmaceutical dosage form by RP-HPLC method.

**Trial-1:**

**Chromatographic Conditions.**

Column : Agilent C18 (4.6*150mm) 5μm
Mobile phase ratio : Water: Methanol (40:60v/v) Detection wavelength : 255nm
Flow rate : 1ml/min Injection volume : 10μl

![Fig 1: Chromatogram of Trial-1](image-url)

**Table 3: Details of Trial-1**

<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>Cobicistat</td>
<td>2.551</td>
<td>8671924</td>
<td>460798</td>
<td>745</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Darunavir</td>
<td>4.879</td>
<td>2283694</td>
<td>179357</td>
<td>1911</td>
<td>2.79</td>
<td>1.45</td>
</tr>
</tbody>
</table>

**Observation:** Darunavir and Cobicistat were separated and two individual peaks are displayed. But they are not clear.

**Trial-2:**
**Chromatographic conditions:**

- **Column**: Thermosil C18 (4.6*150mm) 5μm
- **Mobile phase ratio**: Water: Methanol (40:60% v/v)
- **Detection wavelength**: 255nm
- **Flow rate**: 1ml/min Injection volume 10μl
- **Column temperature**: 40°C
- **Auto sampler temperature**: Ambient

![Fig 2: Chromatogram of Trial 2](image)

<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 Resolutionon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cobicistat</td>
<td>1.828</td>
<td>7913799</td>
<td>394185</td>
<td>722</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Darunavir</td>
<td>3.458</td>
<td>1853381</td>
<td>162758</td>
<td>2614</td>
<td>2.85</td>
<td>1.52</td>
</tr>
</tbody>
</table>

**Observation:**

Peaks symmetry is being improved when compared to the previous trial. Further trials are conducted for better resolution.

**Trial-3:**

**Chromatographic conditions:**

- **Column**: Agilent C18 5μm (4.6*250mm)
- **Mobile phase ratio**: Phosphate buffer (0.05m) pH5.0: Methanol (50:50% v/v)
- **Detection wavelength**: 255nm
- **Flow rate**: 1ml/min
- **Injection volume**: 10μl

![Fig 3: Chromatogram of Trial-3](image)
Table 5: Details of Trial-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>Cobicistat</td>
<td>1.823</td>
<td>9849287</td>
<td>482363</td>
<td>198</td>
<td>1.97</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Darunavir</td>
<td>4.679</td>
<td>3272312</td>
<td>356630</td>
<td>5036</td>
<td>1.15</td>
<td>4.23</td>
</tr>
</tbody>
</table>

**Observation:**
There is noticeable improvement in resolution. But peak symmetry is not achieved.

**Trial-4:**

**Chromatographic conditions:**
Column : Inertsil ODSC185 μm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH4.6:MeOH
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20μl
Auto sampler temperature: Ambient

![Fig 4: Chromatogram of Trial-4](image)

Table 6 : Details of Trial-4

<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>Plate count</th>
<th>Tailing</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cobicistat</td>
<td>3.191</td>
<td>11286305</td>
<td>813690</td>
<td>1587</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Darunavir</td>
<td>3.945</td>
<td>3443649</td>
<td>160557</td>
<td>616</td>
<td>1.80</td>
<td>1.46</td>
</tr>
</tbody>
</table>

**Observation:**
The tailing factor is within the limit. But the other parameters are not within the limit.

**Trial-5:**

**Chromatographic conditions:**
Column : Inertsil C18 5μm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH4.6: CAN (30:70%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20μl Column
Temperature : Ambient
Table 7: 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 count</th>
<th>Plate Tailing</th>
<th>USP Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cobicistat</td>
<td>2.399</td>
<td>946124</td>
<td>155429</td>
<td>5105</td>
<td>1.3</td>
<td>8.1</td>
</tr>
<tr>
<td>2</td>
<td>Darunavir</td>
<td>3.907</td>
<td>111541</td>
<td>13239</td>
<td>3788</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

Observation

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized.

Conclusion

The chromatographic method development for the simultaneous estimation of Cobicistat and Darunavir were optimized by several trials for various parameters as different columns, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Cobicistat and Darunavir in API and pharmaceutical dosage form by RP-HPLC method. Nine trails have been done with various composition of mobile phase. Among them the mobile phase composition acetonitrile, phosphate buffer in the ratio of 70:30 (trail.no.5) using C-18 column as stationary phase and detection at 255nm has produced a well resolved peak.

The results were found to be:
- Theoretical plates: 5105, 3788
- Tailing factor: 1.3, 1.4
- Retention time: 2.399, 3.907
- Peak area: 946124, 111541

Acknowledgements

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References


