Simultaneous Method Development, Validation and Stress Studies of Darunavir and Ritonavir in Bulk and Combined Dosage Form Using UV Spectroscopy

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Abstract

A simple, specific, accurate and precise UV Spectrophotometric method has been developed for the simultaneous estimation of Darunavir and Ritonavir in pharmaceutical dosage form. The absorption maxima of the Darunavir and Ritonavir were found to be 267 nm by using methanol and 240 nm using methanol: water as solvent respectively. Different analytical validation parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines. The accuracy of the method was confirmed by recovery studies of tablet dosage form and was found to be 100% and 99% for Darunavir and Ritonavir respectively. The LOD of Darunavir and Ritonavir was found to be 0.043μg/ml and 0.024μg/ml respectively and LOQ of Darunavir and Ritonavir was found to be 0.132μg/ml and 0.078μg/ml respectively. The developed method was free from interferences due to excipients present in formulation and it can be used for routine quality control analysis.

Keywords: Absorption maxima, analytical, validation parameters, Darunavir, Ritonavir, ICH guidelines.

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INTRODUCTION

Darunavir ethanolate (DRV) is an antiviral drug used in the treatment of human immunodeficiency virus (HIV) protease. It was approved in the year 2006 by the Food and Drug Administration (FDA), it is chemically [(1S,2R)-3-[(4-aminophenyl)sulfonyl][2-methy]propyl]amino]-2-hydroxy-1-(phenylmethyl) propyl]- carbamic acid (3R,3aR,6aR) hexahydrofuro [2,3-b]furan-3-yl ester monoethanolate.

DRV is a second-generation protease inhibitor, it will selectively inhibit the cleavage of HIV-1 encoded Gag- Pol polyproteins in the infected cells, and thereby it prevents the formation of mature virus cells [1-6].

DRV is used with Ritonavir and other medications to treat HIV. It works by slowing the spread of HIV in the body. Ritonavir is an antiretroviral drug from the protease inhibitor class it is used to treat HIV infection and AIDS. The chemical it is (5S,8S, 10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-etraazatridecan-13-oic acid 5-thiazoly methyl ester. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but it will inhibit the same host enzyme that metabolizes other protease inhibitors. This leads to increases in plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy.

From the literature survey it was found that the analytical methods have been reported for the determination of the darunavir in spectrophotometric method and individually in human plasma by liquid chromatography/tandem mass spectrometry, and from the literature survey it was found that Ritonavir estimated by analytical methods such as spectrophotometric methods, reversed phase high performance liquid chromatographic (RP-HPLC) method 7-13, LC-MS14 and HPTLC15 method. Apart from the above, no other methods are also present such as zero and first order derivative spectrophotometric
method was reported for the quantitative determination of (RIT) in pharmaceutical dosage forms. The present work describes the simple precise, accurate and specific method for the simultaneous determination of drugs by UV spectrophotometric method [7-12].

**Introduction to UV spectroscopy**

UV-Visible Spectroscopy is type of absorption Spectroscopy in which light of ultra-violet region (200-400 nm) is absorbed by the molecule.

Principle: UV-Visible Spectroscopy obeys the beer-lamberts law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

This law is expressed as

\[ A = \log \left( \frac{I_0}{I} \right) = \varepsilon cl \]

A=Absorbance.
I_o= Intensity of light upon a sample cell.
I= Intensity of light departing the sample cell.
C= Concentration of the solute.
L=Length of the sample cell and
\( \varepsilon \)=Molar absorptivity [13-18].

**MATERIALS AND METHODS**

**Chemical and reagents**

Darunavir and Ritonavir were obtained as a gift sample from Pharmaceuticals, Hyderabad, telangana. Methanol and other chemicals were analytical grade from Rankem chemicals limited.

**Instrumentation**

Spectroscopic analysis was carried out using Elico SL-210UV/Vis-Double beam spectrophotometer with Spectral treaties software. Spectrophotometer with spectral width 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matching quartz cells was used to measure absorbance of the resulting solutions.

**Selection of common solvent**

After assessing the solubility of drugs in different solvents Methanol has been selected as common solvent for developing spectral characteristics.

**Selection of wavelength**

Weigh accurately about 10 mg of Darunavir in a 10 ml volumetric flask and add methanol until the substance completely dissolves and make the volume up to 10 ml with methanol to obtain the 1000 μg/ml of the component and 10 mg of Ritonavir in a 10 ml volumetric flask and add methanol: water (6:4) and make the volume up to 10 ml with methanol: water in 10 ml volumetric flask to obtain the 1000 μg/ml of the Ritonavir and these both the drugs are further diluted to get 10 μg/ml of Darunavir (standard stock solution A) and 10 μg/ml of Ritonavir (standard stock solution B) in a separate volumetric flask. These dilutions were scanned from 200-400 nm respectively. Wavelengths of Darunavir 267 nm and 240 nm for the Ritonavir respectively and according to the label claim 1; 8 dilutions of DRV and RIT were scanned between 200-400 nm isosbestic points found at 254 nm.
Preparation of standard stock solution
10mg of DRV and RIT were separately weighed in 10 ml volumetric flask and dissolve with methanol for DRV and methanol; water (6: 4) for RIT to get concentration of 1000ppm from this 1 in 10ml was prepared to get the concentration of 100ppm.

Validation parameters
Validation: It is defined as establishing documented evidence which provides a high degree of assurance that a specific process will produce a product meeting its predetermined specifications and quality characteristics.
Linearity
Various standard solutions of Darunavir were prepared by pipetting 0.2 to 2 ml of stock solutions (100 μg/ml) of DAR in 10 ml of volumetric flasks and volume made up to the mark by methanol to obtain the concentrations of 2-20 μg/ml. Then, the standard solutions of Ritonavir were prepared by pipetting 0.5-10 ml from (stock solutions 100 μg/ml) of RTV in 10 ml of volumetric flasks and the volumetric flasks and volume was made up to the mark by methanol: water (6:4) to obtain the final concentrations of 5-100 μg/ml.

Precision
Darunavir (10 ppm) using methanol and RTV (50 ppm) using methanol: water (6:4) as diluents the standard solutions were prepared separately and precision study was performed. This procedure is repeated 6 times and absorbance of all were measured at 267 nm and 240 nm for DAR and RTV. And its %RSD was calculated by using the formula: %RSD = (standard deviation of the measurement / mean value of measurement) x 100 repeated for 6 times and its %RSD was calculated.

Accuracy
The accuracy was determined by spiking the sample matrix of interest with a known concentration of analyte, the recovery studies were carried at three different levels (50, 100 and 150%) on the basis of the label claim. At each level, three determinations were performed simultaneously and percentage recovery was calculated.

Robustness
Small deliberate change in the method are made such as wavelength the determination of the Robustness was performed at +1 nm and -1 nm from the fixed wave length, %RSD was calculated.

Ruggedness
The ruggedness of both the methods was performed by changing analysts and instruments, %RSD were calculated.

LOD & LOQ
LOD is defined as the lowest concentration of an analyte in sample that can be detected, not quantified. LOD = 3.3 σ/S
σ = standard deviation of the response,
S = slope of calibration curve.

LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

LOQ = 10 σ/S
σ = standard deviation of the response,
S = slope of calibration curve.

Analysis of formulation: Simultaneous Equation Method
For the analysis of formulation take the marketed tablet formulation of DURART-R 450 mg (400 mg of Darunavir + 50 mg of Ritonavir).

Weigh the 5 tablets and powder them, Weigh accurately 10 mg equivalent tablet powder in 10 ml of volumetric flask dissolve it in methanol filtered and sonicated for 5 min which gives the stock solution of (1000 ppm), from this take 0.1 ml of the solution in to 10 ml volumetric flask and make up the volume up to 10 ml with methanol (10 ppm), this solution scanned.

Then, the reported amount of Darunavir and Ritonavir was calculated using simultaneous equation method given below:

\[ C_X = \frac{A_2}{A_1} - \frac{A_2 - a_1}{a_2} \frac{a_1}{a_2} - \frac{a_1}{a_2} \]
\[ C_Y = \frac{a_1}{a_2} - \frac{a_1}{a_2} \frac{a_1}{a_2} - \frac{a_1}{a_2} \]

A_1 = Absorbance of formulation at 267 nm.
A_2 = Absorbance of formulation at 240 nm.
a_1 = Absorptivity of DRV at 267 nm.
a_2 = Absorptivity of DRV at 240 nm.
a_1 = Absorptivity of RIT at 267 nm.
a_2 = Absorptivity of RIT at 240 nm.
C_X = Concentration of Darunavir.
C_Y = Concentration of Ritonavir.

Forced Degradation Studies
Forced degradation studies are also known as stress testing. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule.

Objective for forced Degradation Studies
- To recognize the chemical properties of drug molecules.
- To elucidate the structure of degradation products.
- To resolve stability-related problems.
- To establish the stability of a drug substance in the formulation.
- To reveal the degradation mechanisms of the drug substance and drug product.
- To distinguish degradation products that is related to drug products.

ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, Q1B exemplify the forced degradation studies

ICH Q1A: stability testing of new drug substances and products
ICH Q1B: photo stability testing of new drug substances and products [19-21].
**Procedure**

**Acid Degradation**
From 10ppm of drug solution, taken 1 ml of the 10ppm solution into 10ml volumetric flask +1 ml of 0.1 N HCl kept for 24 hours. After 24 hours neutralize with 1 ml of 0.1N NaOH Measured its absorbance at 251nm.

**Alkali Degradation**
From 10ppm of drug solution, taken 1 ml of 10ppm solution into 10 ml volumetric flask +1 ml of 0.1N NaOH Was kept for 24 hours. After 24 hours neutralize with 1 ml of 0.1 N HCl Measured its absorbance at 251nm.

**Photolytic Degradation**
10mg of drug was exposed to UV light in UV chamber for 3hrs by placing the drug in Petri dish. After 3hrs Sample was diluted to get concentration of 10 µg/ml and absorbance was measured at 251nm.

**Thermal Degradation**
Drug was exposed to dry heat 40°C in oven at for 3hrs by placing the drugs in Petri dish. Weighed 10mg of drug and diluted to get a final concentration of 10 µg/ml Measure the absorbance at 251nm and calculate the percentage of Degradation.

**RESULTS AND DISCUSSION**

![Calibration Plot](image1)

**Fig-6: Calibration curve of Darunavir**

![Calibration Plot](image2)

**Fig-7: Calibration curve of Ritonavir**

![Calibration Plot](image3)

**Fig-8: calibration plot of combined drugs**
Table-1: precision data

| S. No | Darunavir (10ppm) 267nm | Ritonavir (50ppm) 240nm | Combined Drugs 254nm (1:8) |
|-------|------------------------|-------------------------|--------------------------|
| 1     | 0.5147                 | 0.5780                   | 0.6076                   |
| 2     | 0.5145                 | 0.5781                   | 0.6075                   |
| 3     | 0.5140                 | 0.5782                   | 0.6074                   |
| 4     | 0.5135                 | 0.5780                   | 0.6075                   |
| 5     | 0.5145                 | 0.5781                   | 0.6074                   |
| 6     | 0.5157                 | 0.5782                   | 0.6073                   |
| Mean  | 0.5144                 | 0.5781                   | 0.6074                   |
| SD    | 0.0007386              | 0.0000894                | 0.000104                 |
| %RSD  | 0.143%                 | 0.0321%                  | 0.0172%                  |

Table-2: Accuracy data of Darunavir

| % level | Combined concentration | DRV concentration | %Recovery | Mean% Recovery |
|---------|------------------------|-------------------|-----------|----------------|
| 50%     | 1:8                    | 2ppm              | 99.1%     | 99.6%          |
| 100%    | 1:8                    | 4ppm              | 99.2%     | 99.3%          |
| 150%    | 1:8                    | 6ppm              | 100.1%    | 100%           |

Table-3: Accuracy data of Ritonavir

| % level | Combined concentration | RIT Concentration | %Recovery | % Mean Recovery |
|---------|------------------------|-------------------|-----------|----------------|
| 50%     | 1:8                    | 5ppm              | 99.1%     | 99.2%          |
| 100%    | 1:8                    | 10ppm             | 99.1%     | 99.3%          |
| 150%    | 1:8                    | 15ppm             | 99%       | 100%           |

Table-4: Robustness data of Darunavir

| S. No | 266nm | 268nm |
|-------|-------|-------|
| 1     | 0.5152| 0.5189|
| 2     | 0.5152| 0.5188|
| 3     | 0.5150| 0.5188|
| Mean  | 0.5151| 0.5188|
| SD    | 0.000154| 0.000057|
| %RSD  | 0.0298%| 0.0111%|

Table-5: Robustness data of Ritonavir

| S.NO | 239nm | 241nm |
|------|-------|-------|
| 1    | 0.5691| 0.5792|
| 2    | 0.5653| 0.5796|
| 3    | 0.5670| 0.5802|
| Mean | 0.5671| 0.5796|
| SD   | 0.00190| 0.000503|
| %RSD | 0.711%| 0.1800%|
### Table-6: Robustness data of combined drugs

| S. No | 253nm  | 255nm  |
|-------|--------|--------|
| 1     | 0.5932 | 0.6154 |
| 2     | 0.5936 | 0.6156 |
| 3     | 0.5935 | 0.6154 |
| Mean  | 0.5934 | 0.6156 |
| SD    | 0.000208 | 0.0002 |
| %RSD  | 0.0350% | 0.0324% |

### Table-7: Ruggedness data of darunavir

| S. No | Absorbance (Abs) | Analyst-1 | Analyst-2 |
|-------|------------------|-----------|-----------|
| 1     | 0.5086           | 0.5069    |           |
| 2     | 0.5089           | 0.5073    |           |
| 3     | 0.5080           | 0.5078    |           |
| Mean  | 0.5085           | 0.5073    |           |
| SD    | 0.000458         | 0.000450  |           |
| %RSD  | 0.09%            | 0.0887%   |           |

### Table-8: Ruggedness data of Ritonavir

| S. No | Absorbance (Abs) | Analyst-1 | Analyst-2 |
|-------|------------------|-----------|-----------|
| 1     | 0.5691           | 0.5792    |           |
| 2     | 0.5653           | 0.5796    |           |
| 3     | 0.5673           | 0.5802    |           |
| Mean  | 0.5672           | 0.5796    |           |
| SD    | 0.000190         | 0.0005033 |           |
| %RSD  | 0.0334%          | 0.0868%   |           |

### Table-9: Ruggedness data of combined drugs

| S. No | Absorbance (Abs) | Analyst-1 | Analyst-2 |
|-------|------------------|-----------|-----------|
| 1     | 0.6132           | 0.6025    |           |
| 2     | 0.6131           | 0.6025    |           |
| 3     | 0.6132           | 0.6024    |           |
| 4     | 0.6133           | 0.6023    |           |
| 5     | 0.6132           | 0.6024    |           |
| 6     | 0.6131           | 0.6024    |           |
| Mean  | 0.6131           | 0.6025    |           |
| SD    | 0.0000752        | 0.0000816 |           |
| %RSD  | 0.0122%          | 0.0135%   |           |

### Table-10: LOD&LOQ data of Darunavir and Ritonavir

|       | LOD  | LOQ   |
|-------|------|-------|
| DRV   | 0.043μg/ml | 0.132μg/ml |
| RIT   | 0.024μg/ml | 0.078μg/ml |

### Table-10: Analysis of formulation by simultaneous method

| Drugs name     | Concentrations |
|----------------|----------------|
| Darunavir Cₓ  | 8.8μg/ml       |
| Ritonavir Cᵧ  | 1.1μg/ml       |
Table-11: stress studies

| Type of Degradation          | Darunavir | Ritonavir |
|------------------------------|-----------|-----------|
| Acid Degradation (0.1N HCL)  | 19.1%     | 15%       |
| Alkali Degradation (0.1N NaOH)| 19%   | 17%       |
| Photolytic Degradation       | 17%       | 18.5%     |
| (UV Chamber)                 |           |           |
| Peroxide Degradation         | 19.6%     | 17.8%     |
| (3%H$_2$O$_2$)               |           |           |
| Thermal Degradation          | 15%       | 16.4%     |
| (Hot air Oven-40 c)          |           |           |

Fig-9: Stress studies of Darunavir and Ritonavir

CONCLUSION

The developed UV spectrophotometric method is simple, precise, accurate, linear, and reproducible for the estimation of Darunavir and Ritonavir in combined dosage form without any interference from the excipients. It can be successfully applied for the routine analysis of both the drugs in pharmaceutical dosage form.

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