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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF DARUNAVIR AND RITONAVIR IN COMBINED TABLET FORMULATION

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ABSTRACT:
A simple, precise, accurate, and cost-effective reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Darunavir and Ritonavir in combined tablet dosage form. The separation was carried out using a Thermo ODS C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.01N KH_2PO_4 buffer (pH 3.1) and acetonitrile in the ratio of 40:60 v/v. The flow rate was 1.0 mL/min and detection was performed at 260 nm. The retention times of Darunavir and Ritonavir were found to be 2.6 and 3.4 minutes, respectively. The method exhibited good linearity in the concentration range of 30–180 µg/mL for Darunavir and 5–30 µg/mL for Ritonavir, with correlation coefficients of 0.999 for both. The percentage recoveries for Darunavir and Ritonavir were 99.06% and 98.68%, respectively. The method was validated as per ICH Q2 (R1) guidelines for accuracy, precision, specificity, linearity, robustness, LOD, and LOQ. The proposed method can be effectively applied for routine quality control analysis of Darunavir and Ritonavir in pharmaceutical formulations.
Keywords: Darunavir, Ritonavir, RP-HPLC, Method Development, Validation, ICH Guidelines

INTRODUCTION

HIV (Human Immunodeficiency Virus) infection remains one of the major global health issues, with millions of people requiring lifelong antiretroviral therapy. Darunavir and Ritonavir are protease inhibitors used in combination therapy for the treatment of HIV-1 infection. Darunavir is a second-generation HIV-1 protease inhibitor, notable for its ability to bind to and inhibit both wild-type and resistant forms of HIV protease. Ritonavir, originally developed as an antiretroviral agent, is now predominantly used as a pharmacokinetic enhancer because of its potent inhibitory effect on cytochrome P450 3A4 (CYP3A4), thereby increasing plasma concentrations of co-administered protease inhibitors like Darunavir.

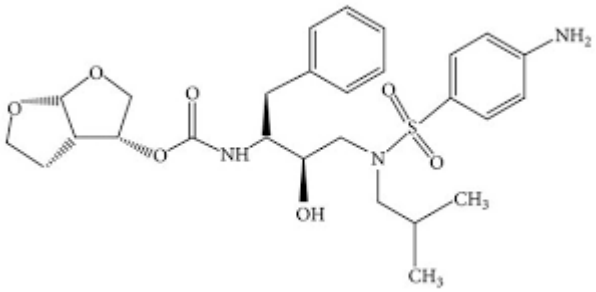

Fig.1 Chemical structure of Darunavir

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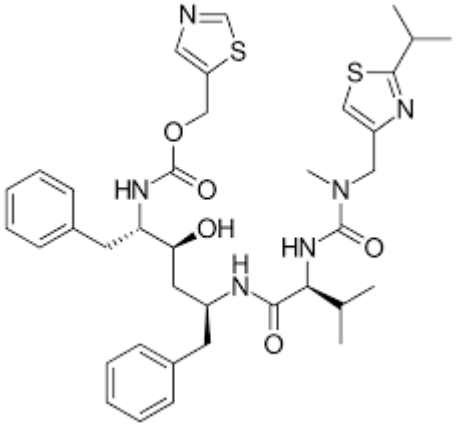

Fig2. Chemical Structure of Ritonavir

Combination therapies involving Darunavir and Ritonavir offer potent viral suppression and improved resistance profiles. However, due to the complex nature of fixed-dose combination tablets and the potential interactions between active pharmaceutical ingredients (APIs) and excipients, it becomes imperative to employ a robust, precise, and validated analytical method to simultaneously determine the concentrations of both drugs in dosage forms. A validated RP-HPLC method is essential not only for routine quality control but also for stability studies, bioequivalence evaluations, and regulatory compliance.

Numerous analytical methods have been reported for the individual estimation of Darunavir and Ritonavir, including UV spectrophotometry, HPTLC, LC-MS/MS, and HPLC. However, very few methods are reported for the simultaneous estimation of both drugs in combined dosage forms, particularly in a cost-effective and time-efficient manner suitable for industrial application. Hence, the development of a simple, economical, and robust RP-HPLC method for the simultaneous quantification of Darunavir and Ritonavir in tablet dosage form becomes a necessity.

In this study, a reversed-phase HPLC method was developed using a Thermo ODS C18 column and a mobile phase comprising phosphate buffer and acetonitrile. The method was optimized for critical chromatographic parameters such as retention time, resolution, peak symmetry, and theoretical plates. Further, the method was validated in accordance with the International Council for Harmonisation (ICH) guidelines Q2(R1) for various parameters including accuracy, precision, linearity, robustness, specificity, limit of detection (LOD), and limit of quantification (LOQ).

This research aims to provide a validated analytical tool for the simultaneous estimation of Darunavir and Ritonavir in fixed-dose combination tablets that is not only reliable and reproducible but also efficient for use in routine quality control laboratories and pharmaceutical manufacturing units.

Materials and Methods

Chemicals and Reagents

Darunavir and Ritonavir reference standards were procured from a certified pharmaceutical manufacturer. A commercially available fixed-dose combination tablet (Virem-R) containing Darunavir (600 mg) and Ritonavir (100 mg) was used as the test formulation. Analytical-grade chemicals including potassium dihydrogen phosphate (KH_2PO_4), orthophosphoric acid (OPA), methanol, and acetonitrile (all HPLC grade) were used throughout the study. Milli-Q water was used for the preparation of buffer and diluent. All solvents and solutions were filtered through 0.45 µm nylon membrane filters and degassed using sonication before use.

Preparation of Buffer Solution

A buffer solution was prepared by dissolving 1.36 g of KH_2PO_4 in 1000 mL of Milli-Q water. The pH was adjusted to 3.1 using dilute orthophosphoric acid. The solution was filtered and degassed before use.

Preparation of Diluent

A 1:1 v/v mixture of acetonitrile and water was used as the diluent for both standard and sample preparations.

Standard Stock Solution Preparation

Accurately weighed 120 mg of Darunavir and 20 mg of Ritonavir were transferred into a 100 mL volumetric flask. Approximately 75 mL of diluent was added and the solution was sonicated for 10 minutes to ensure complete dissolution. The volume was made up to 100 mL with diluent. From this solution, 1 mL was further diluted to 10 mL with diluent to obtain a working solution containing 120 µg/mL of Darunavir and 20 µg/mL of Ritonavir.

Sample Preparation

Twenty tablets were weighed and finely powdered. A quantity equivalent to the average weight of one tablet was transferred into a 100 mL volumetric flask, and about 70 mL of diluent was added. The mixture was sonicated for 25 minutes to extract the drugs and then brought to volume with the same diluent. The solution

was filtered, and 0.2 mL of the filtrate was diluted to 10 mL with diluent to obtain a sample solution containing 120 µg/mL of Darunavir and 20 µg/mL of Ritonavir.

Method Validation

The method was validated as per ICH Q2(R1) guidelines for parameters including system suitability, linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System Suitability

System suitability was evaluated by injecting six replicates of the standard solution. Parameters such as retention time, peak area, tailing factor, theoretical plates, and %RSD were calculated to ensure system performance was within acceptable limits.

Linearity

Linearity was assessed by analyzing six concentrations ranging from 30–180 µg/mL for Darunavir and 5–30 µg/mL for Ritonavir. Calibration curves were plotted as peak area versus concentration, and regression equations along with correlation coefficients (r^2) were calculated.

Precision

Precision was evaluated through intra-day (repeatability) and inter-day (intermediate precision) studies. Six replicate injections were performed on the same day and on two different days. %RSD was calculated for both drugs to assess method consistency.

Accuracy

Accuracy was assessed by recovery studies at three concentration levels: 50%, 100%, and 150%. Known amounts of standard were spiked into pre-analyzed sample solutions, and the percentage recovery was calculated.

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve using the following formulas:

$$\mathbf{LOD = 3.3 \times (\sigma/S),\ LOQ = 10 \times (\sigma/S)}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Robustness

Robustness of the method was evaluated by making deliberate changes in chromatographic conditions, including ±0.1 mL/min in flow rate, ±2% in mobile phase composition, and ±2°C in column temperature. The impact of these changes was assessed based on system suitability parameters.

Specificity

Specificity was demonstrated by injecting blank, placebo, standard, and sample solutions. The absence of interfering peaks at the retention times of Darunavir and Ritonavir confirmed the specificity of the method.

Assay

The assay was performed by comparing the peak areas of the test sample with those of the standard solution. The analysis was conducted in triplicate, and the amount of each drug present in the formulation was calculated and expressed as a percentage of the labelled claim.

Results and Discussion

The primary objective of this study was to develop a simple, precise, accurate, and cost-effective RP-HPLC method for the simultaneous estimation of Darunavir and Ritonavir in fixed-dose combination tablets. The method was successfully optimized and validated in accordance with ICH Q2(R1) guidelines. Results from method development trials and subsequent validation experiments are discussed below.

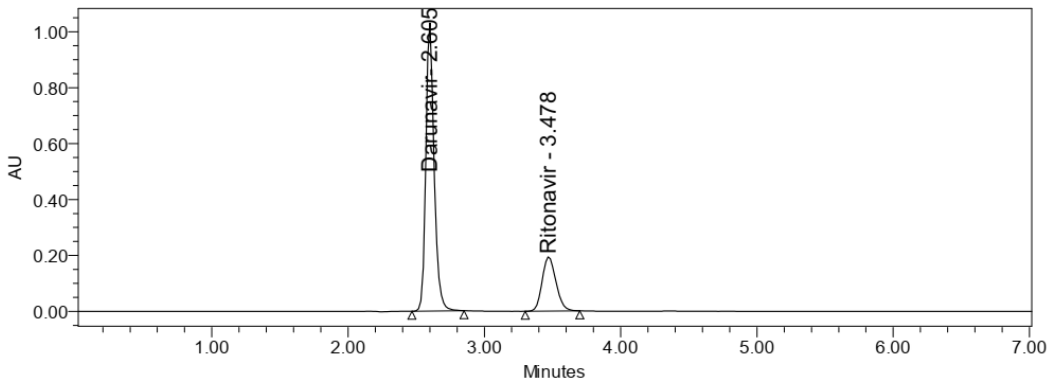

Fig 3. Optimized chromatogram of Darunavir and Ritonavir

Instrumentation and Chromatographic Conditions

The analysis was performed using a WATERS HPLC 2965 system equipped with a PDA detector and auto-sampler. Data acquisition and processing were performed using Empower 2 software. Separation was achieved

on a Thermo ODS C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of a mixture of 0.01N KH_2PO_4 buffer (pH 3.1 adjusted with OPA) and acetonitrile in the ratio of 40:60 v/v. The flow rate was maintained at 1.0 mL/min, the detection wavelength was set at 260 nm, the column temperature was maintained at 30°C, and the injection volume was 10 µL. The run time for each sample was approximately 6 minutes.

System Suitability

System suitability testing ensures that the chromatographic system is working effectively and can produce reliable results. The parameters assessed included retention time (Rt), tailing factor, and theoretical plates. Darunavir eluted at 2.6 minutes, while Ritonavir eluted at 3.4 minutes, indicating a well-resolved separation. The tailing factor was 0.74 for Darunavir and 1.1 for Ritonavir, which demonstrates excellent peak symmetry. Theoretical plate numbers were 5486 and 4251, respectively, exceeding the minimum requirement of 2000, thereby confirming column efficiency and system performance.

Linearity

The method showed excellent linearity over the concentration ranges of 30–180 µg/mL for Darunavir and 5–30 µg/mL for Ritonavir. Calibration curves were constructed by plotting the peak areas against respective concentrations. The correlation coefficients (r^2) were found to be 0.999 for both drugs, confirming a strong linear relationship. The regression equations were:

- Darunavir: y = 20879x + 4078.3
- Ritonavir: y = 40536x + 16956

  These values indicate the method's capability to provide consistent responses over a broad concentration range.

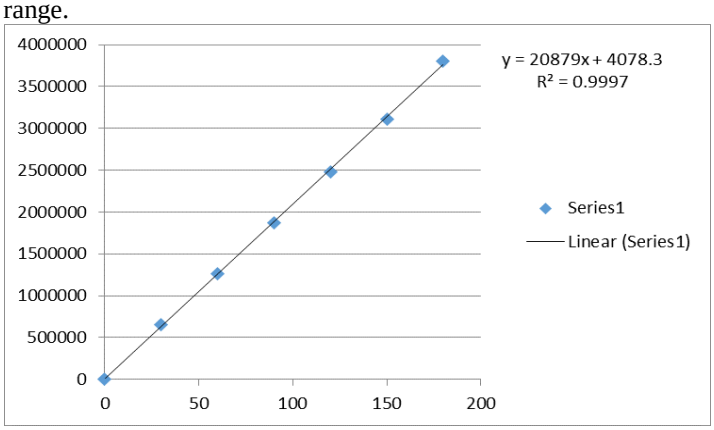

Fig: 4 Calibration curve of Darunavir

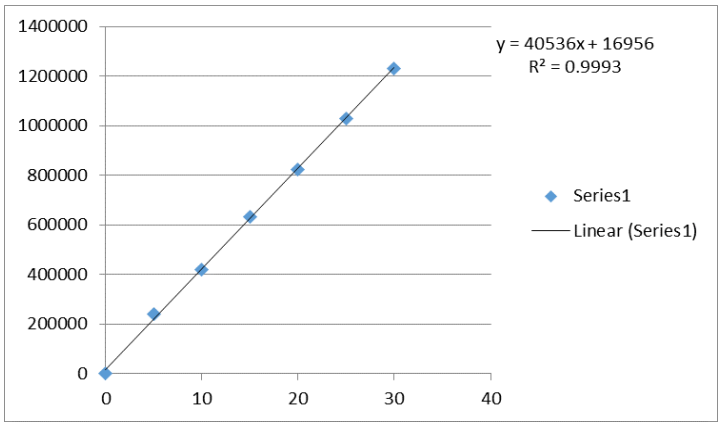

Fig: 5 Calibration curve of Ritonavir

Precision

Precision was evaluated through both repeatability (intra-day) and intermediate precision (inter-day). For repeatability, six injections of the same sample solution were analyzed within a single day, yielding %RSD values of 0.37% for Darunavir and 0.93% for Ritonavir. For intermediate precision, the same procedure was repeated on the following day, resulting in %RSD values of 0.91% and 0.88% for Darunavir and Ritonavir, respectively. All values were below the acceptable threshold of 2%, confirming the reproducibility of the method.

Accuracy (Recovery Studies)

The accuracy of the method was determined by recovery studies conducted at three concentration levels: 50%, 100%, and 150% of the target concentration. Each concentration level was tested in triplicate. The average

percentage recovery was found to be 99.06% for Darunavir and 98.68% for Ritonavir, with individual values ranging from 96.5% to 101.3%. These results indicate that the method is capable of recovering the analytes accurately without interference from excipients or other components of the formulation.

Table: 1. Accuracy results of Darunavir And Ritonavir

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	Avg
Darunavir	60	57.9	96.5	99.06
	120	119.25	99.375	
	180	182.35	101.3056	
Ritonavir	10	9.8	98.0	98.68
	20	19.88	99.4	
	30	29.6	98.6	

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the standard deviation of the response and the slope of the calibration curve. The LOD for Darunavir was 0.27 µg/mL, and the LOQ was 0.81 µg/mL. For Ritonavir, the LOD was 0.8 µg/mL and the LOQ was 2.4 µg/mL. These low values reflect the high sensitivity of the method, making it suitable for detecting trace levels of both drugs.

Robustness

The robustness of the method was evaluated by making deliberate minor changes to method parameters, including flow rate (±0.1 mL/min), mobile phase composition (±2%), and temperature (±2°C). The method remained unaffected by these changes, as indicated by %RSD values that remained well within acceptable limits (<2%). This confirms that the method is robust and reliable under varied analytical conditions.

Specificity

Specificity was demonstrated by analyzing blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times of Darunavir and Ritonavir, confirming the method's ability to selectively quantify both drugs in the presence of excipients and other formulation components.

Assay of Tablet Formulation

The assay of the commercial formulation was conducted by comparing the peak areas of sample and standard solutions. The content of Darunavir and Ritonavir in the tablet formulation was found to be 99.56% and 100.12% of the label claim, respectively. These results fall within the acceptable range (98–102%), confirming the accuracy and suitability of the developed method for routine quality control analysis.

Conclusion

The developed RP-HPLC method is simple, sensitive, accurate, and robust for simultaneous estimation of Darunavir and Ritonavir in tablet dosage forms. The method satisfies ICH validation criteria and can be successfully applied for quality control in pharmaceutical industries.

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