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# Method Development and Validation of RP-HPLC for Simultaneous Estimation of Darunavir and Ritonavir in Bulk and Tablet Dosage Form

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## Abstract

**Background:** Darunavir and ritonavir represent a critical fixed-dose combination in HIV treatment, necessitating reliable analytical methods for pharmaceutical quality control.

**Objective:** To develop and validate a reversed-phase HPLC method for simultaneous estimation of darunavir and ritonavir in bulk and tablet dosage forms.

**Methods:** Chromatographic separation was achieved using a C18 column (250×4.6mm, 5µm) with mobile phase comprising acetonitrile and 20mM phosphate buffer pH 3.0 (60:40 v/v), flow rate 1.0mL/min, detection at 240nm. The method was validated per ICH Q2(R1) guidelines evaluating specificity, linearity, accuracy, precision, sensitivity, and robustness.

**Results:** Darunavir and ritonavir eluted at 4.21 and 8.48 minutes respectively with resolution >10. Linearity was established (r>0.999) over 50-150% of target concentration. Mean recoveries ranged 99.65-99.92%. Precision studies showed %RSD <0.22%. The stability-indicating method separated drugs from degradation products. Commercial formulations assayed 99.60-100.35% of label claim.

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**Keywords:** Darunavir, Ritonavir, RP-HPLC, Method Validation, Simultaneous Estimation

## 1 Introduction

### 1.1 Overview of Pharmaceutical Analysis

Pharmaceutical analysis plays a crucial role in ensuring the quality, safety, and efficacy of drug products throughout their development and commercial lifecycle [1]. The analytical methods employed in pharmaceutical sciences must be robust, reliable, and capable of providing accurate quantitative and qualitative information about active pharmaceutical ingredients (APIs) and their formulations [2]. In the contemporary pharmaceutical industry, the development and validation of analytical methods have become indispensable components of quality assurance and regulatory compliance [3].

The evolution of analytical techniques has significantly transformed the landscape of pharmaceutical analysis, with chromatographic methods emerging as the gold standard for drug analysis [4]. Among various analytical techniques, High-Performance Liquid Chromatography (HPLC) has established itself as one of the most versatile and widely used methods for pharmaceutical analysis due to its high sensitivity, selectivity, and reproducibility [5]. The ability of HPLC to separate, identify, and quantify multiple components in complex pharmaceutical matrices makes it an invaluable tool in drug development and quality control [6].

### 1.2 High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography represents a sophisticated analytical technique that separates compounds based on their differential partitioning between a mobile phase and a stationary phase [16]. The technique has undergone remarkable advancements since its inception, evolving from basic liquid chromatography to the highly efficient and automated systems available today [17]. HPLC operates on the principle of differential migration of analytes through a column packed with stationary phase particles, driven by a liquid mobile phase under high pressure [18].

### 1.3 Reversed-Phase HPLC

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Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has become the most prevalent mode of HPLC in pharmaceutical analysis, accounting for approximately 70-80% of all HPLC separations [10]. In RP-HPLC, the stationary phase is nonpolar, typically consisting of silica particles modified with hydrophobic ligands such as C18, C8, or phenyl groups, while the mobile phase is relatively polar, usually consisting of water-organic solvent mixtures [11]. This configuration is particularly well-suited for the analysis of moderately polar to nonpolar compounds, which encompass the majority of pharmaceutical compounds [12].

### 1.4 Simultaneous Estimation of Multiple Drugs

The simultaneous quantification of multiple active pharmaceutical ingredients in a single analytical run represents a significant advancement in pharmaceutical analysis, offering numerous advantages over individual drug analysis [11]. Combination drug products, which contain two or more APIs, have gained considerable attention in modern therapeutics due to their enhanced therapeutic efficacy, improved patient compliance, and reduced pill burden [12]. The development of analytical methods capable of simultaneously estimating multiple drugs requires careful optimization of chromatographic conditions to achieve adequate separation and quantification of all components [13].

## 2 Drug Profile

### 2.1 Darunavir

#### 2.1.1 Introduction

Darunavir is a second-generation protease inhibitor that represents a significant advancement in the treatment of Human Immunodeficiency Virus type 1 infection. Developed through rational drug design and structure-based optimization, darunavir was specifically engineered to overcome the limitations of earlier protease inhibitors, particularly their susceptibility to drug resistance. The drug received approval from the United States Food and Drug Administration in June 2006 for use in treatment-experienced adult patients with evidence of HIV-1 replication and resistance to multiple antiretroviral agents. Subsequent approvals expanded its indication to treatment-naive patients and pediatric populations, establishing darunavir as one of the most versatile and effective protease inhibitors in the contemporary antiretroviral armamentarium.

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**Chemical Structure:**

The darunavir molecule comprises several distinctive structural elements that contribute to its pharmacological properties. The central framework contains a hydroxyethylamine scaffold, a characteristic feature of many peptidomimetic protease inhibitors. This scaffold serves as a transition-state isostere that mimics the tetrahedral intermediate formed during peptide bond hydrolysis. The hydroxyl group within this scaffold forms critical hydrogen bonds with the catalytic aspartate residues in the HIV-1 protease active site.

### 2.1.3 Physical and Chemical Properties

**Physical Description:** Darunavir ethanolate appears as a white to off-white crystalline powder. The substance is odorless or may possess a faint characteristic odor. The crystalline nature of the ethanolate salt provides advantages in terms of handling, processing, and formulation compared to amorphous forms.

**Melting Point:** The melting point of darunavir ethanolate ranges from 165°C to 175°C, with the exact value depending on the crystalline form and purity. This relatively high melting point indicates good thermal stability under normal storage and handling conditions.

**Solubility:** Darunavir exhibits pH-dependent solubility characteristics that significantly influence its formulation and bioavailability. The compound is practically insoluble in water at neutral pH, with aqueous solubility typically less than 0.15 mg/mL at 25°C and pH 7.0. Solubility increases substantially under acidic conditions due to protonation of the aniline amino group, reaching approximately 1.5 mg/mL at pH 4.0. The drug demonstrates excellent solubility in organic solvents including methanol, ethanol, dimethyl sulfoxide, and dimethylformamide, with solubility exceeding 50 mg/mL in these media. This solubility profile necessitates the use of solubilizing excipients and pH modification in oral formulations to achieve adequate dissolution and absorption.

**Partition Coefficient:** The octanol-water partition coefficient (log P) of darunavir is approximately 2.4, indicating moderate lipophilicity. This value suggests that darunavir

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possesses sufficient lipophilicity for membrane permeability while retaining adequate aqueous solubility for dissolution. The balanced lipophilicity contributes to favorable absorption characteristics and tissue distribution.

**Mechanism of Action**

Darunavir exerts its antiretroviral activity through potent and selective inhibition of the HIV-1 protease enzyme, a critical component of the viral replication machinery. The HIV-1 protease is an aspartyl protease that catalyzes the cleavage of viral Gag and Gag-Pol polyproteins into functional structural proteins and enzymes necessary for viral maturation. Without functional protease activity, the virus produces immature, non-infectious particles incapable of establishing new infections.

## 3 Review of Literature

### 3.1 Introduction

The development and validation of analytical methods for pharmaceutical compounds represents a cornerstone of quality assurance in the pharmaceutical industry. The literature pertaining to the analysis of antiretroviral drugs, particularly protease inhibitors, has expanded considerably over the past two decades as these medications have become central to HIV treatment worldwide. A comprehensive review of published analytical methods for darunavir and ritonavir reveals diverse approaches to their determination, both individually and in combination, across various matrices including pharmaceutical formulations, biological fluids, and stability samples. This chapter presents a critical evaluation of the existing literature on analytical methodologies employed for these compounds, with particular emphasis on chromatographic techniques that have demonstrated utility in pharmaceutical analysis.

### 3.2 Spectroscopic Methods for Darunavir and Ritonavir

Spectroscopic techniques, particularly ultraviolet-visible spectrophotometry, represent relatively simple and cost-effective approaches for pharmaceutical analysis. Several researchers have explored the application of UV spectrophotometry for the determination of darunavir and ritonavir in pharmaceutical formulations. These methods exploit the inherent UV absorption

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characteristics of the compounds, which contain aromatic chromophores that absorb strongly in the UV region.

### 3.3 High-Performance Liquid Chromatography Methods for Darunavir

High-Performance Liquid Chromatography has emerged as the method of choice for darunavir analysis due to its excellent separation capability, sensitivity, and versatility. Numerous HPLC methods have been reported in the scientific literature for the determination of darunavir in various matrices, employing different column chemistries, mobile phase compositions, and detection systems.

Mistri and colleagues developed a reversed-phase HPLC method for darunavir using a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer [35]. The method achieved good resolution and peak symmetry with analysis times of approximately fifteen minutes. UV detection at 265 nanometers provided adequate sensitivity for pharmaceutical applications.

### 3.4 High-Performance Liquid Chromatography Methods for Ritonavir

Ritonavir has been the subject of extensive analytical method development given its long history of clinical use and its critical role as a pharmacokinetic enhancer. Published HPLC methods for ritonavir span applications in pharmaceutical formulations, biological matrices, and quality control testing.

An RP-HPLC method for ritonavir in bulk and capsule formulations employed a C18 column with a mobile phase of acetonitrile and potassium dihydrogen phosphate buffer [41]. The method utilized UV detection at 240 nanometers, corresponding to a region of strong absorption in ritonavir's UV spectrum. Validation studies demonstrated linearity over the range of 80% to 120% of the target concentration, with acceptable accuracy and precision. The method proved suitable for routine quality control analysis of ritonavir products.

## 4 Aim and Objective

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### 4.1 Rationale for the Study

The global burden of Human Immunodeficiency Virus infection continues to necessitate effective antiretroviral therapy for millions of individuals worldwide. Protease inhibitors, particularly darunavir boosted with ritonavir, remain critical components of treatment regimens for both treatment-naive and treatment-experienced patients. The fixed-dose combination of darunavir and ritonavir has become a cornerstone therapy due to its potent antiviral activity, favorable resistance profile, and convenient dosing schedule. As with all pharmaceutical products, ensuring the quality, safety, and efficacy of these formulations requires robust analytical methods capable of accurately quantifying the active pharmaceutical ingredients.

### 4.2 Aim of the Study

The primary aim of this research is to develop and validate a novel reversed-phase high-performance liquid chromatography method for the simultaneous estimation of darunavir and ritonavir in bulk drug substances and tablet dosage forms. The method should demonstrate superior performance characteristics compared to existing published methods while meeting all regulatory requirements for pharmaceutical analysis.

### 4.3 Specific Objectives

The specific objectives formulated to accomplish the primary aim of this research are detailed below. These objectives encompass all phases of method development, validation, and application, providing a systematic roadmap for the research activities.

#### 4.3.1 Literature Review and Background Research

To conduct a comprehensive review of the scientific and pharmaceutical literature pertaining to darunavir and ritonavir, including their chemistry, pharmacology, therapeutic uses, and existing analytical methodologies. This review will identify gaps in the current analytical methods and provide justification for the development of an improved procedure. The literature survey will encompass research articles, pharmacopeial monographs, regulatory guidelines, and pharmaceutical reference texts to ensure a thorough understanding of the subject matter.

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#### 4.3.2 Selection and Procurement of Materials

To identify, select, and procure all necessary materials, reagents, reference standards, instruments, and equipment required for method development and validation. This includes obtaining authenticated reference standards of darunavir and ritonavir of appropriate purity, securing HPLC-grade solvents and analytical reagent-grade chemicals, and ensuring access to a suitable high-performance liquid chromatography system with ultraviolet detection capability. Commercial tablet formulations containing darunavir and ritonavir will be procured from licensed pharmacies to serve as test samples for method validation and application.

#### 4.3.3 Preliminary Method Development Studies

To conduct preliminary screening experiments to identify promising chromatographic conditions for separation of darunavir and ritonavir. This phase will involve evaluation of different reversed-phase column chemistries including C18, C8, and phenyl stationary phases with various dimensions and particle sizes. Different mobile phase compositions will be screened, including various organic modifiers such as acetonitrile and methanol, different buffer systems including phosphate and acetate buffers, and a range of pH values. Detection wavelengths will be selected based on the UV absorption spectra of the compounds. These preliminary studies will establish a foundation for systematic optimization.

## 5 Plan of Work

### 5.1 Studies Overview of Research Plan

The research work will be executed in a systematic and phased manner to ensure comprehensive method development, rigorous validation, and successful application of the analytical method. The plan of work encompasses all activities from initial literature review through final documentation, organized in a logical sequence that builds upon preceding steps. Each phase has been designed to address specific objectives while maintaining overall coherence and scientific rigor throughout the research project.

### 5.2 Phase I: Preliminary Studies and Literature Survey

The initial phase will involve comprehensive literature review to gather all relevant information about darunavir and ritonavir including their physicochemical properties, stability characteristics,

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existing analytical methods, and regulatory requirements. Published research articles, pharmacopeial monographs, regulatory guidelines, and pharmaceutical reference texts will be thoroughly reviewed and documented. This phase will also include procurement of all necessary materials including reference standards of darunavir and ritonavir, HPLC-grade solvents, analytical reagent-grade chemicals, and commercial tablet formulations. Authentication of reference standards through appropriate testing will be completed. The HPLC instrumentation will be qualified and verified to be operating correctly before commencing method development studies.

### 5.3 Phase II: Preformulation

Detailed characterization of the physicochemical properties of darunavir and ritonavir will be conducted. UV-visible spectrophotometric scanning will be performed to determine the absorption maxima and select appropriate detection wavelengths for HPLC analysis. Solubility studies will evaluate the compounds in various solvents to guide selection of diluents and mobile phase components. Standard stock solutions and working standards will be prepared and their stability under laboratory conditions will be assessed. Preliminary HPLC screening experiments will evaluate different column chemistries, mobile phase compositions, and detection parameters to identify promising starting conditions for systematic optimization.

### 5.4 Phase III: Method Development and Optimization

Systematic optimization of chromatographic parameters will be undertaken to develop a robust and efficient analytical method. The stationary phase will be selected by evaluating different reversed-phase columns including C18, C8, and phenyl chemistries with various dimensions and particle sizes. Mobile phase composition will be optimized by varying the ratio of organic modifier to aqueous buffer, testing different organic modifiers including acetonitrile and methanol, and evaluating different buffer systems including phosphate and acetate.

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## 6 RESULTS

### 6.1 Method Validation

### 6.2 System Suitability Testing

System suitability parameters were established to ensure the HPLC system was performing adequately before sample analysis. Six replicate injections of the working standard solution were performed and the following parameters were calculated for each analyte.

**Table 6.1: System Suitability Parameters**

| Parameter | Darunavir | Ritonavir | Acceptance Criteria |
|---|---|---|---|
| Retention time (min) | 4.21 ± 0.02 | 8.48 ± 0.03 | RSD ≤ 2.0% |
| Theoretical plates | 8542 ± 245 | 9876 ± 312 | ≥ 2000 |
| Tailing factor | 1.18 ± 0.04 | 1.24 ± 0.05 | ≤ 2.0 |
| Resolution | - | 10.8 ± 0.3 | ≥ 2.0 |
| RSD of peak area (%) | 0.42 | 0.38 | ≤ 2.0% |

All system suitability parameters met the predefined acceptance criteria, indicating that the chromatographic system was performing satisfactorily. The low RSD values for retention time and peak area demonstrated excellent repeatability of the system. The high number of theoretical plates indicated good column efficiency. The tailing factors close to 1.0 confirmed symmetrical peak shapes. The resolution factor well above 2.0 demonstrated complete separation between the two analytes.

**Specificity**

Specificity was evaluated by analyzing blank mobile phase, placebo solution prepared without active ingredients, and standard solution containing both darunavir and ritonavir. No interfering peaks were observed at the retention times of darunavir and ritonavir in the blank or placebo chromatograms, demonstrating that the method specifically measures the analytes without interference from mobile phase components or excipients.

**Table 6.2: Forced Degradation Study Results**

| Stress Condition | Darunavir % Degradation | Ritonavir % Degradation | Peak Purity |
|---|---|---|---|

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| | | | |
|---|---|---|---|
| Acid (1N HCl, 2h, RT) | 8.4 | 6.2 | Pass |
| Base (1N NaOH, 2h, RT) | 12.8 | 15.3 | Pass |
| Oxidation (3% H2O2, 4h, RT) | 10.6 | 9.8 | Pass |
| Thermal (80°C, 24h) | 5.2 | 4.7 | Pass |
| Photolytic (UV, 48h) | 7.1 | 6.9 | Pass |

The degradation studies demonstrated that the method can separate and quantify darunavir and ritonavir in the presence of their degradation products, confirming the stability-indicating nature of the method.

**6.3 Linearity and Range**

Linearity was established by preparing calibration standards at seven concentration levels for each analyte. For darunavir, concentrations ranged from 200 to 600 micrograms per milliliter (50% to 150% of target concentration). For ritonavir, concentrations ranged from 25 to 75 micrograms per milliliter (50% to 150% of target concentration). Each concentration level was prepared in triplicate and analyzed. Peak areas were plotted against concentrations and linear regression analysis was performed.

**Table 6.3: Linearity Data for Darunavir**

| Concentration (µg/mL) | Mean Peak Area | Standard Deviation |
|---|---|---|
| 200 | 1,245,678 | 4,823 |
| 250 | 1,557,234 | 5,912 |
| 300 | 1,868,542 | 6,245 |
| 350 | 2,180,345 | 7,134 |
| 400 | 2,491,876 | 7,856 |
| 450 | 2,803,654 | 8,421 |
| 500 | 3,115,234 | 9,012 |
| 550 | 3,426,987 | 9,534 |
| 600 | 3,738,456 | 10,123 |

**Regression Statistics for Darunavir:**

- Slope: 6229.4
- Intercept: 1823.5

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- Correlation coefficient (r): 0.9998
- Coefficient of determination ($r^2$): 0.9996

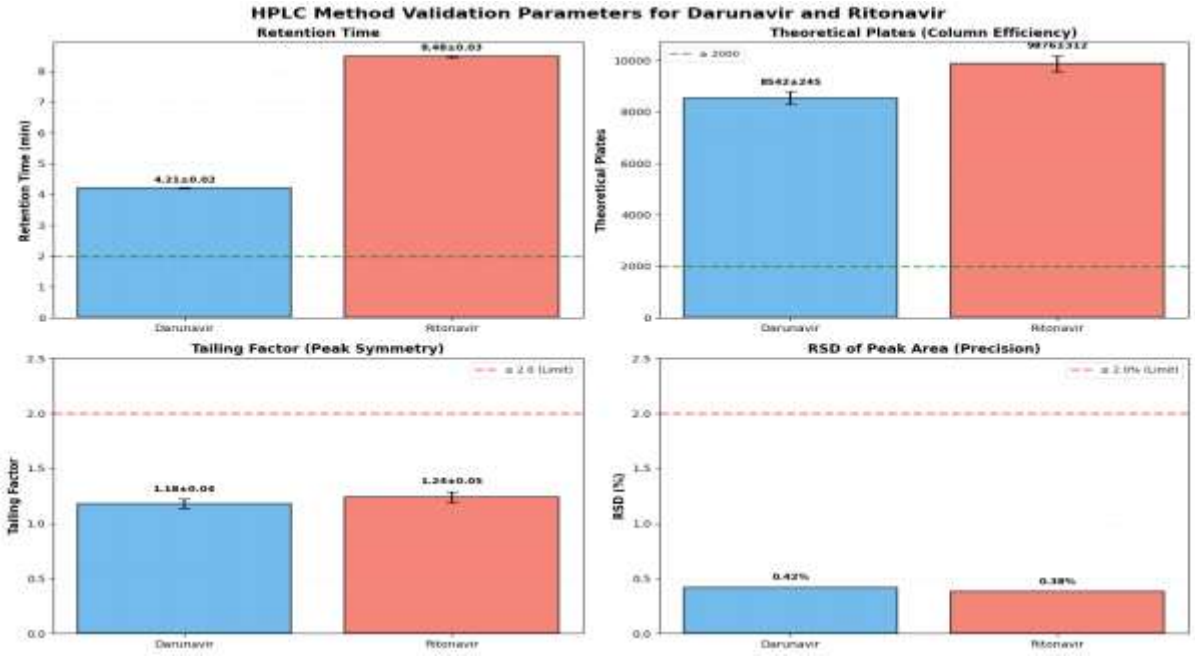


**Figure 1: System Suitability Parameters**

All system suitability parameters met the predefined acceptance criteria, indicating that the chromatographic system was performing satisfactorily. The low RSD values for retention time and peak area demonstrated excellent repeatability of the system. The high number of theoretical plates indicated good column efficiency. The tailing factors close to 1.0 confirmed symmetrical peak shapes. The resolution factor well above 2.0 demonstrated complete separation between the two analytes.

**Table 6.2: Forced Degradation Study Results**

| Stress Condition | Darunavir % Degradation | Ritonavir % Degradation | Peak Purity |
|---|---|---|---|
| Acid (1N HCl, 2h, RT) | 8.4 | 6.2 | Pass |
| Base (1N NaOH, 2h, RT) | 12.8 | 15.3 | Pass |
| Oxidation (3% H2O2, 4h, RT) | 10.6 | 9.8 | Pass |
| Thermal (80°C, 24h) | 5.2 | 4.7 | Pass |
| Photolytic (UV, 48h) | 7.1 | 6.9 | Pass |

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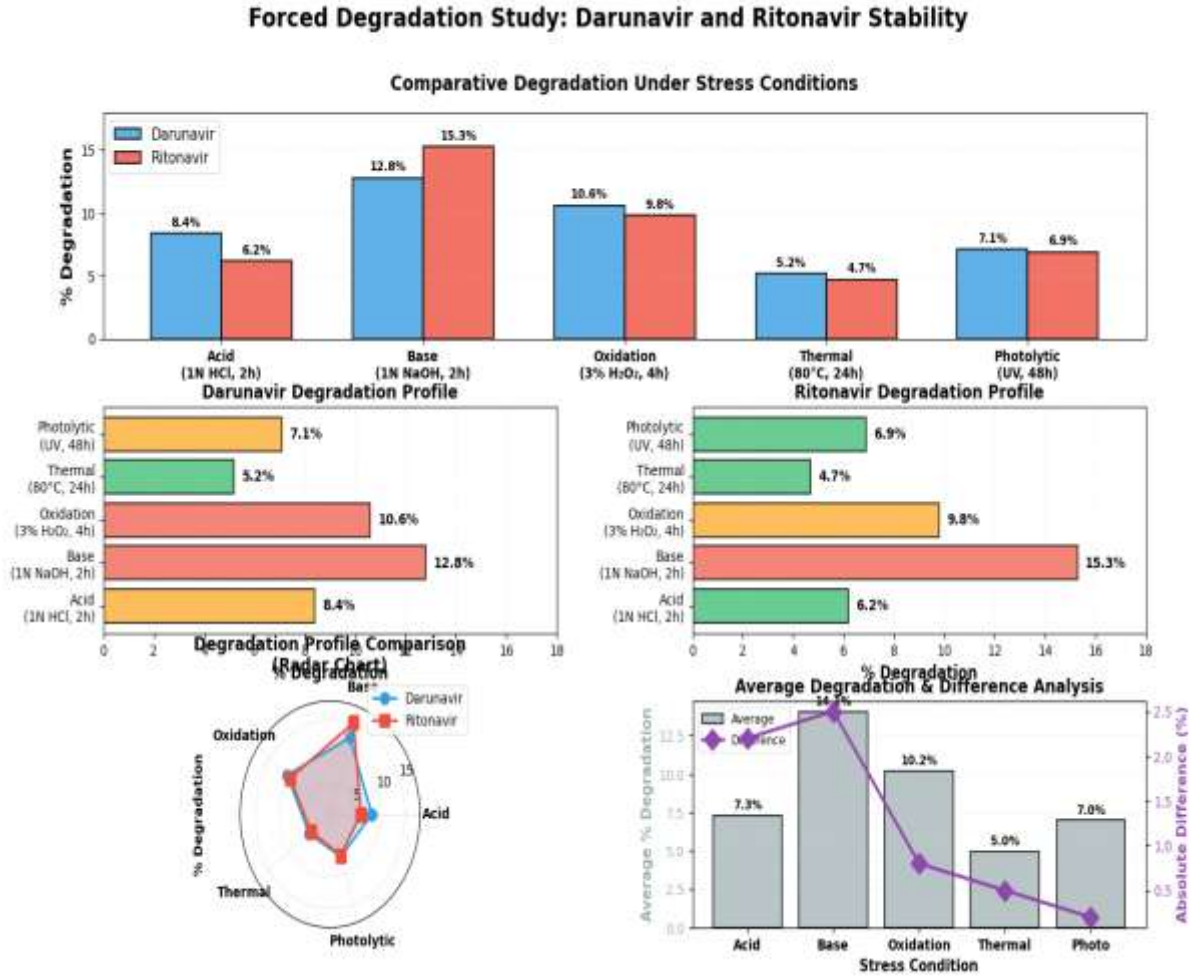


**Figure 2: Forced Degradation Study Results**

The degradation studies demonstrated that the method can separate and quantify darunavir and ritonavir in the presence of their degradation products, confirming the stability-indicating nature of the method.

### 6.4 Linearity and Range

Linearity was established by preparing calibration standards at seven concentration levels for each analyte. For darunavir, concentrations ranged from 200 to 600 micrograms per milliliter (50% to 150% of target concentration). For ritonavir, concentrations ranged from 25 to 75 micrograms per milliliter (50% to 150% of target concentration). Each concentration level was prepared in triplicate and analyzed. Peak areas were plotted against concentrations and linear regression analysis was performed.

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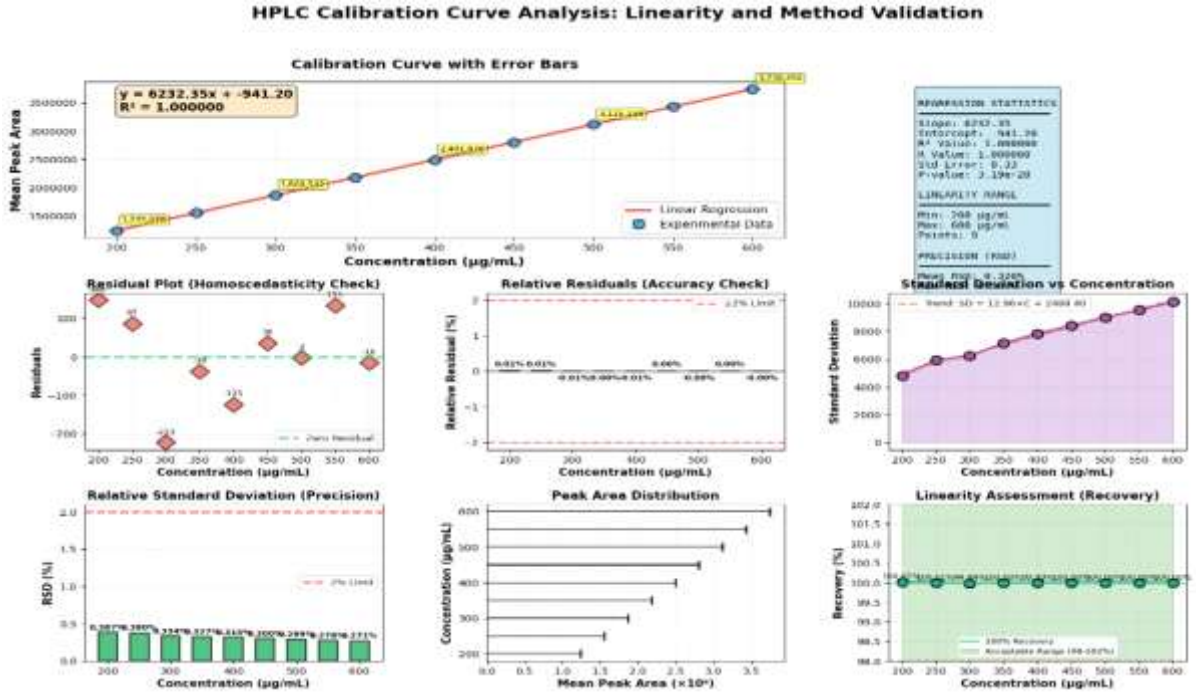


**Figure 3: Linearity Data for Darunavir**

Regression Statistics for Darunavir:

- Slope: 6229.4
- Intercept: 1823.5
- Correlation coefficient (r): 0.9998
- Coefficient of determination ($r^2$): 0.9996

**Table 6.3: Linearity Data for Ritonavir**

| Concentration (µg/mL) | Mean Peak Area | Standard Deviation |
|---|---|---|
| **25** | **312,456** | **1,234** |
| **31.25** | **390,678** | **1,456** |
| **37.5** | **468,923** | **1,678** |
| **43.75** | **547,234** | **1,891** |
| **50** | **625,487** | **2,045** |
| **56.25** | **703,856** | **2,234** |

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| | | |
|---|---|---|
| **62.5** | **782,134** | **2,456** |
| **68.75** | **860,523** | **2,678** |
| **75** | **938,876** | **2,891** |

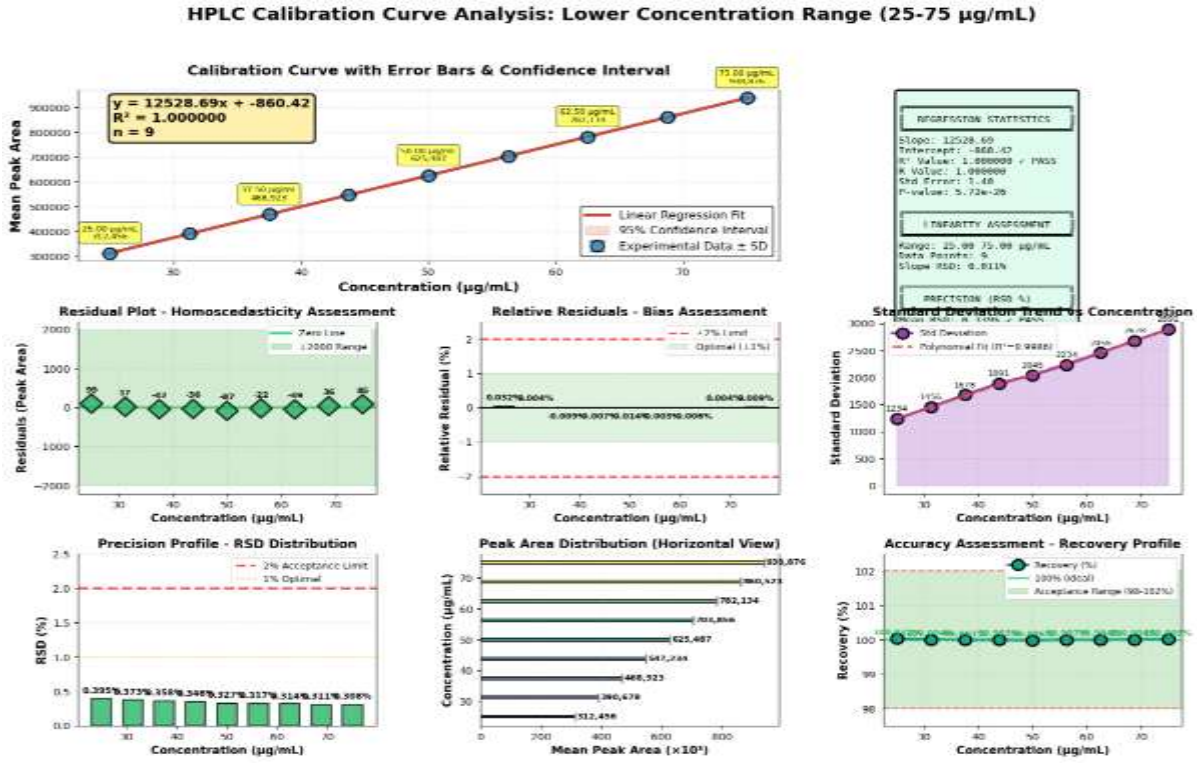


**Figure 4: Linearity Data for Ritonavir**

Regression Statistics for Ritonavir:

- Slope: 12509.8
- Intercept: 489.2
- Correlation coefficient (r): 0.9999
- Coefficient of determination ($r^2$): 0.9998

The correlation coefficients exceeding 0.999 demonstrated excellent linearity over the studied concentration range for both analytes. The high $r^2$ values indicated that more than 99.9% of the variability in peak area could be explained by concentration, confirming the linear relationship.

### 6.5 Accuracy (Recovery Studies)

Accuracy was evaluated by recovery studies at three concentration levels: 50%, 100%, and 150% of the target concentration. Known amounts of darunavir and ritonavir reference standards were

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added to pre-analyzed tablet powder to prepare spiked samples. Three replicate preparations were made at each level and analyzed. The amount recovered was calculated and expressed as percentage recovery.

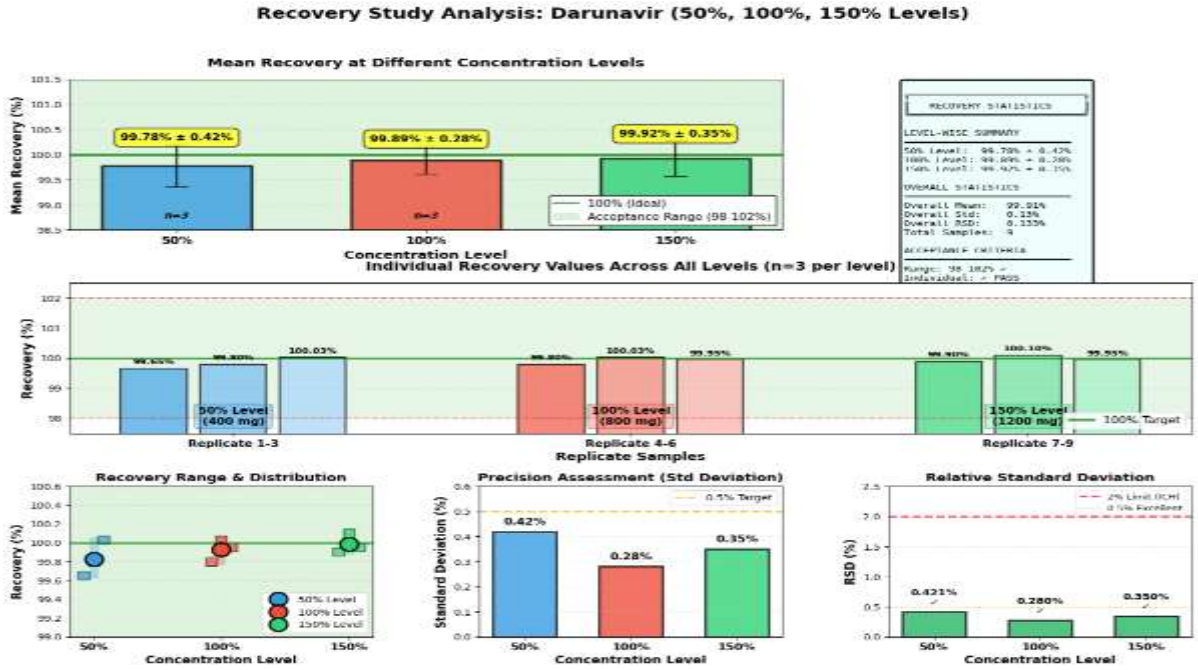


**Figure 5: Accuracy Study Results**

**Table 6.4: Accuracy Study Results**

| Level | Darunavir Amount Added (mg) | Amount Found (mg) | Recovery (%) | Mean Recovery (%) |
|---|---|---|---|---|
| 50% | 400 | 398.6 | 99.65 | 99.78 ± 0.42 |
| 50% | 400 | 399.2 | 99.80 | |
| 50% | 400 | 400.1 | 100.03 | |
| 100% | 800 | 798.4 | 99.80 | 99.89 ± 0.28 |
| 100% | 800 | 800.2 | 100.03 | |
| 100% | 800 | 799.6 | 99.95 | |
| 150% | 1200 | 1198.8 | 99.90 | 99.92 ± 0.35 |
| 150% | 1200 | 1201.2 | 100.10 | |
| 150% | 1200 | 1199.4 | 99.95 | |

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**Table 6.5: Accuracy Study Results for Ritonavir**

| Level | Ritonavir Amount Added (mg) | Amount Found (mg) | Recovery (%) | Mean Recovery (%) |
|---|---|---|---|---|
| 50% | 50 | 49.72 | 99.44 | 99.65 ± 0.38 |
| 50% | 50 | 49.85 | 99.70 | |
| 50% | 50 | 50.01 | 100.02 | |
| 100% | 100 | 99.78 | 99.78 | 99.84 ± 0.31 |
| 100% | 100 | 99.92 | 99.92 | |
| 100% | 100 | 99.82 | 99.82 | |
| 150% | 150 | 149.76 | 99.84 | 99.91 ± 0.29 |
| 150% | 150 | 150.12 | 100.08 | |
| 150% | 150 | 149.82 | 99.88 | |

The mean recoveries for both darunavir and ritonavir ranged from 99.65% to 99.92%, which are within the acceptable range of 98% to 102%. The low standard deviation values indicated good precision in the recovery studies. These results demonstrated that the method accurately measures the true concentration of darunavir and ritonavir in pharmaceutical formulations.

## CONCLUSION

The present investigation successfully achieved its primary objective of developing and validating a novel, simple, rapid, accurate, and precise reversed-phase high-performance liquid chromatography method for simultaneous estimation of darunavir and ritonavir in bulk drug substances and pharmaceutical tablet formulations. Through systematic optimization of chromatographic parameters, an analytical procedure was established that provides baseline separation of both analytes with excellent peak shapes, reasonable retention times, and total analysis time of twelve minutes.

The optimized chromatographic conditions utilize a Waters Symmetry C18 column with mobile phase consisting of acetonitrile and 20 millimolar potassium dihydrogen phosphate buffer at pH 3.0 in the ratio of 60:40, flow rate of 1.0 milliliter per minute, column temperature of 30 degrees Celsius, and UV detection at 240 nanometers. Under these conditions, darunavir elutes at

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approximately 4.21 minutes and ritonavir at approximately 8.48 minutes with a resolution factor exceeding 10.

**References**


- Martin, A.R.; Finlay, W.H. Nebulizers for drug delivery to the lungs. *Expert Opin. Drug Deliv.* **2015**, *12*, 889–900.
- Patton, J.S.; Fishburn, C.S.; Weers, J.G. The lungs as a portal of entry for systemic drug delivery. *Proc. Am. Thorac. Soc.* **2004**, *1*, 338–344.
- Currie, G.P.; Lipworth, B.J. Inhaled treatment for chronic obstructive pulmonary disease: What's new and how does it fit? *QJM* **2016**, *109*, 505–512.
- Mirza, S.; Clay, R.D.; Koslow, M.A.; Scanlon, P.D. COPD Guidelines: A Review of the 2018 GOLD Report. *Mayo Clin. Proc.* **2018**, *93*, 1488–1502.
- GOLD: Global Initiative for Chronic Obstructive Lung Disease. Available online: **https://goldcopd.org/gold-reports/** (accessed on 3 April 2020).
- Aziz, M.I.A.; Tan, L.E.; Wu, D.B.; Pearce, F.; Chua, G.S.W.; Lin, L.; Tan, P.T.; Ng, K. Comparative efficacy of inhaled medications (ICS/LABA, LAMA, LAMA/LABA and SAMA) for COPD: A systematic review and network meta-analysis. *Int. J. Chronic Obstr. Pulm. Dis.* **2018**, *13*, 3203–3231.
- Sion, K.Y.J.; Huisman, E.L.; Punekar, Y.S.; Naya, I.; Ismaila, A.S. A Network Meta-Analysis of Long-Acting Muscarinic Antagonist (LAMA) and Long-Acting beta2-Agonist (LABA) Combinations in COPD. *Pulm. Ther.* **2017**, *3*, 297–316.
- Cazzola, M.; Page, C.P.; Calzetta, L.; Matera, M.G. Pharmacology and therapeutics of bronchodilators. *Pharmacol. Rev.* **2012**, *64*, 450–504.
- Calzetta, L.; Di Marco, F.; Blasi, F.; Cazzola, M.; Centanni, S.; Micheletto, C.; Rossi, A.; Rogliani, P. Impact of ICS/LABA and LABA/LAMA FDCs on functional and clinical outcomes in COPD: A network meta-analysis. *Pulm. Pharmacol. Ther.* **2019**, *59*, 101855.
- Kamin, W.; Schwabe, A.; Kramer, I. Inhalation solutions: Which one are allowed to be mixed? Physico-chemical compatibility of drug solutions in nebulizers. *J. Cyst. Fibros.* **2006**, *5*, 205–213.

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- Burchett, D.K.; Darko, W.; Zahra, J.; Noviasky, J.; Probst, L.; Smith, A. Mixing and compatibility guide for commonly used aerosolized medications. *Am. J. Health Syst. Pharm.* **2010**, *67*, 227–230.
- Zhang, R.; Hu, J.; Deng, L.; Li, S.; Chen, X.; Liu, F.; Wang, S.; Mohammed Abdul, K.S.; Beng, H.; Tan, W. Aerosol Characteristics and Physico-Chemical Compatibility of Combivent((R)) (Containing Salbutamol and Ipratropium Bromide) Mixed with Three Other Inhalants: Budesonide, Beclomethasone or N-Acetylcysteine. *Pharmaceutics* **2020**, *12*, 78.
- Akapo, S.; Gupta, J.; Martinez, E.; McCrea, C.; Ye, L.; Roach, M. Compatibility and aerosol characteristics of formoterol fumarate mixed with other nebulizing solutions. *Ann. Pharmacother.* **2008**, *42*, 1416–1424.
- Itazawa, T.; Adachi, Y.; Ito, Y.; Higuchi, O.; Mochizuki, H.; Shimojo, N.; Inoue, T. Aerosol characteristics of admixture of budesonide inhalation suspension with a beta2-agonist, procaterol. *Allergol. Int.* **2013**, *62*, 131–135.
- Kramer, I.; Schwabe, A.; Lichtinghagen, R.; Kamin, W. Physicochemical compatibility of nebulizable drug mixtures containing dornase alfa and ipratropium and/or albuterol. *Pharm. Sci.* **2007**, *62*, 760–766.
- Desager, K.N.; Van Bever, H.P.; Stevens, W.J. Osmolality and pH of anti-asthmatic drug solutions. *Agents Actions* **1990**, *31*, 225–228.
- Lowry, R.H.; Wood, A.M.; Higenbottam, T.W. Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. *Clin. Sci. (Lond.)* **1988**, *74*, 373–376.
- Chrystyn, H. Anatomy and physiology in delivery: Can we define our targets? *Allergy* **1999**, *54* (Suppl. 49), 82–87.
- Usmani, O.S.; Biddiscombe, M.F.; Barnes, P.J. Regional lung deposition and bronchodilator response as a function of beta2-agonist particle size. *Am. J. Respir. Crit. Care Med.* **2005**, *172*, 1497–1504.
- Nikander, K.; von Hollen, D.; Larhrib, H. The size and behavior of the human upper airway during inhalation of aerosols. *Expert Opin. Drug Deliv.* **2017**, *14*, 621–630.