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Research

Development and validation of a stability-indicating rp-hplc method for simultaneous estimation of rilpivirine and cabotegravir in dosage forms

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Abstract

A simple, accurate, and precise method for the simultaneous estimation of Rilpivirine and Cabotegravir in pharmaceutical dosage forms was developed and validated using RP-HPLC. The analysis was performed on a Sunfire C18 column (150 mm x 4.6 mm, 5 µm) with a mobile phase of 0.1% orthophosphoric acid and acetonitrile in a 60:40 ratio at a flow rate of 1.0 mL/min. Retention times for Rilpivirine and Cabotegravir were 2.174 min and 2.815 min, respectively. Method validation demonstrated linearity, accuracy, and precision, with %RSD values below 2%, recovery rates of 99.51%-100.35%, and LOD/LOQ values of 0.38 µg/mL/1.15 µg/mL for Rilpivirine and 0.06 µg/mL/0.19 µg/mL for Cabotegravir. This stability-indicating method is efficient and economical for routine quality control in pharmaceutical industries.

Keywords: Rilpivirine, Cabotegravir, RP-HPLC, Method Validation, Stability-Indicating

INTRODUCTION

The quality of a drug plays an essential role in ensuring its safety and efficacy. Quality assurance and control of pharmaceutical and chemical formulations are critical to providing safe and effective drug formulations to consumers. Analysis of pure drug substances and their pharmaceutical dosage forms is pivotal in assessing their suitability for patient use. The reliability of analytical data depends on the quality of the methods employed in generating this data. Hence, the development of robust and rugged analytical methods is crucial for the statutory certification of drugs and their formulations with regulatory authorities. The quality and safety of a drug are generally assured by effectively monitoring and controlling both the assay and impurities. While the assay determines the drug's potency, impurities determine its safety. Assaying pharmaceutical products plays a significant role in ensuring their efficacy for patients. Developing methods for different drugs poses numerous challenges depending on their nature and properties. Researchers face the task of achieving selectivity, speed, cost-effectiveness, simplicity, sensitivity, reproducibility, and accuracy in results, all of which are essential for creating new analytical methods suitable for adoption by the pharmaceutical industry and chemical laboratories.

Various physico-chemical methods are utilized to study the physical phenomena resulting from chemical reactions. Among these, optical methods (refractometry, polarimetry, emission and fluorescence analysis), photometry (UV-visible, IR spectroscopy, and nepheloturbidimetry), and chromatographic methods (column, paper, thin layer, gas-liquid, and high-performance liquid chromatography) are prominent. Emerging techniques like nuclear magnetic resonance (NMR), paramagnetic resonance (PMR), and the combination of mass spectroscopy with gas chromatography have become increasingly significant. These advancements address the need for new analytical methods to control the quality of the constantly growing number of new drugs. Modern pharmaceutical analysis must fulfil requirements such as minimizing time consumption, meeting pharmacopeial accuracy demands, ensuring economical analysis, and providing precise and selective methods.

MATERIALS AND METHODS

Materials

Rilpivirine and Cabotegravir pure drugs (API), Combination Rilpivirine and Cabotegravir formulation (**Cabenuva**), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instrumentation and Chromatographic Conditions

The analysis was conducted on a WATERS HPLC 2695 system equipped with a photodiode array detector. Chromatography was performed using a Sunfire C18 column (150 x 4.6 mm, 5 µm) at a column temperature of 30°C, with UV detection at 250 nm.

Mobile phase and Sample preparation

The mobile phase used for HPLC typically consists of mixtures of organic solvents and water or aqueous buffers. It is crucial to ensure that the drug remains stable in the mobile phase throughout the analysis. High salt concentrations should be avoided as they can lead to precipitation and damage the equipment. To reduce costs and toxicity, methanol is often preferred over acetonitrile when feasible. Additionally, care should be taken to minimize buffer absorbance, as certain acids like trifluoroacetic or formic acid absorb strongly at shorter wavelengths, potentially interfering with detection. Alternatives such as phosphoric acid are recommended for these cases.

For sample preparation, the diluent is chosen based on the solubility of the drugs, such as a mixture of acetonitrile and HPLC-grade water in a 50:50 ratio. For buffer preparation, a 0.1% ortho-phosphoric acid (OPAB) solution is made by diluting 1 mL of concentrated OPAB to 1000 mL with water. Standard stock solutions are prepared by accurately weighing 60 mg of Rilpivirine and 40 mg of Cabotegravir, transferring them to a 100 mL volumetric flask, adding ¾ of the diluent, sonicating for 10 minutes, and making up the volume with the diluent. This results in concentrations of 600 µg/mL for Rilpivirine and 400 µg/mL for Cabotegravir. To prepare standard working solutions, 1 mL of the stock solution is diluted to 10 mL with the diluent, resulting in concentrations of 60 µg/mL for Rilpivirine and 40 µg/mL for Cabotegravir. For sample stock solutions, 1 mL of the injection vial, equivalent to 300 mg of Rilpivirine and 200 mg of Cabotegravir, is transferred into a 250 mL volumetric flask. To this, 100 mL of diluent is added, the solution is sonicated for 25 minutes, made up to volume with diluent, and filtered. Finally, sample working solutions are prepared by diluting 0.5 mL of the filtered sample stock solution to 10 mL with the diluent, yielding final concentrations of 60 µg/mL for Rilpivirine and 40 µg/mL for Cabotegravir.

Validation Parameters

The method was validated as per ICH guidelines, covering specificity, linearity, precision, accuracy, LOD, LOQ, and robustness. Forced degradation studies under stress conditions were performed to ensure the method's stability-indicating capability.

RESULTS AND DISCUSSION

System Suitability and Specificity

Retention times for Rilpivirine and Cabotegravir were consistent, with %RSD values <2%, indicating system suitability. No interfering peaks were observed, confirming specificity.

Linearity and Precision

The method exhibited excellent linearity for Rilpivirine (15-90 µg/mL) and Cabotegravir (10-60 µg/mL) with correlation coefficients of 0.999. Intra-day and inter-day precision studies showed %RSD <2%.

Accuracy and Recovery

Nephrolithiasis, or kidney stones, represents a multifaceted global health concern with a rising incidence that underscores the need for enhanced diagnostic accuracy and improved recovery strategies. Advanced imaging techniques, including low-dose computed tomography (CT) scans and ultrasonography, have significantly improved the precision of kidney stone detection. These methodologies enable clinicians to characterize the size, location, and composition of renal calculi, fostering timely and appropriate treatment decisions. Equally transformative are advancements in minimally invasive surgical techniques, such as ureteroscopy, laser lithotripsy, and percutaneous nephrolithotomy. These approaches have set new standards in patient care by reducing recovery times, minimizing procedural complications, and improving overall outcomes. The integration of robotics and enhanced imaging during these procedures has further enhanced surgical precision and efficacy, exemplifying the evolution of nephrolithiasis management. Recovery protocols have also witnessed notable progress, with an increasing emphasis on multimodal analgesia and enhanced recovery after surgery (ERAS) pathways. These strategies not only mitigate postoperative pain and dependency on opioids but also facilitate a quicker return to normal activities, thereby improving patient satisfaction and long-term quality of life.

In summary, the convergence of diagnostic advancements, innovative surgical techniques, and patient-centered recovery protocols is reshaping the landscape of nephrolithiasis management. These developments promise not only more accurate and less invasive treatment options but also a holistic approach to recovery, addressing the physical and psychological well-being of affected individuals.

LOD AND LOQ

LOD values for Rilpivirine and Cabotegravir were 0.38 µg/mL and 0.06 µg/mL, respectively. LOQ values were 1.15 µg/mL for Rilpivirine and 0.19 µg/mL for Cabotegravir.

Robustness and Stability-Indicating Capability

The method was robust against deliberate variations in chromatographic conditions. Forced degradation studies revealed no interference with drug peaks, confirming the method's stability-indicating nature.

Method development

Method development was done by changing various, mobile phase ratios, buffers etc.

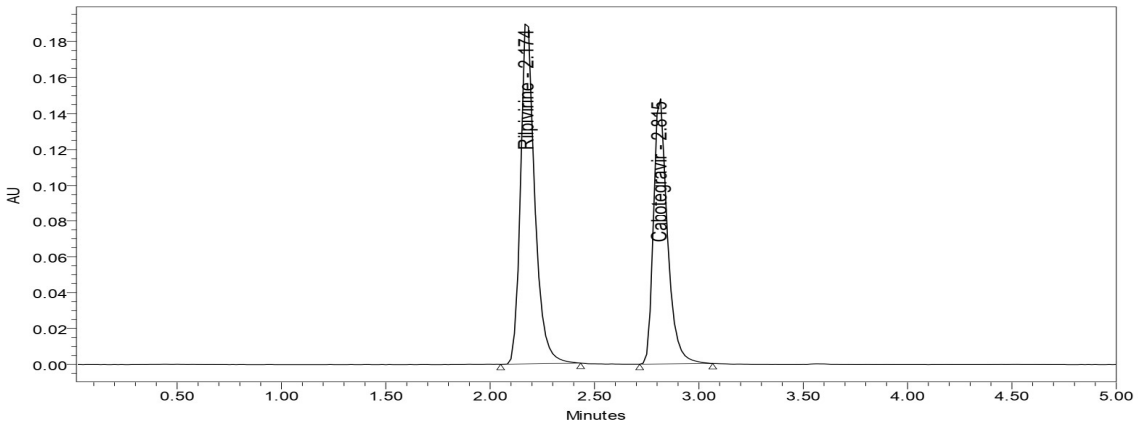

Fig 1: Optimized Chromatogram

Rilpivirine and Cabotegravir were eluted at 2.174 min and 2.815 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table 1: System suitability parameters for Rilpivirine and Cabotegravir

S no	Rilpivirine			Cabotegravir			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.174	5145	1.29	2.807	8748	1.36	5.2
2	2.178	5278	1.26	2.814	8765	1.32	5.2

3	2.181	5656	1.27	2.815	9426	1.31	5.2
4	2.188	5521	1.29	2.820	9079	1.33	5.2
5	2.190	5362	1.32	2.822	8914	1.35	5.2
6	2.190	5113	1.29	2.825	8799	1.35	5.2

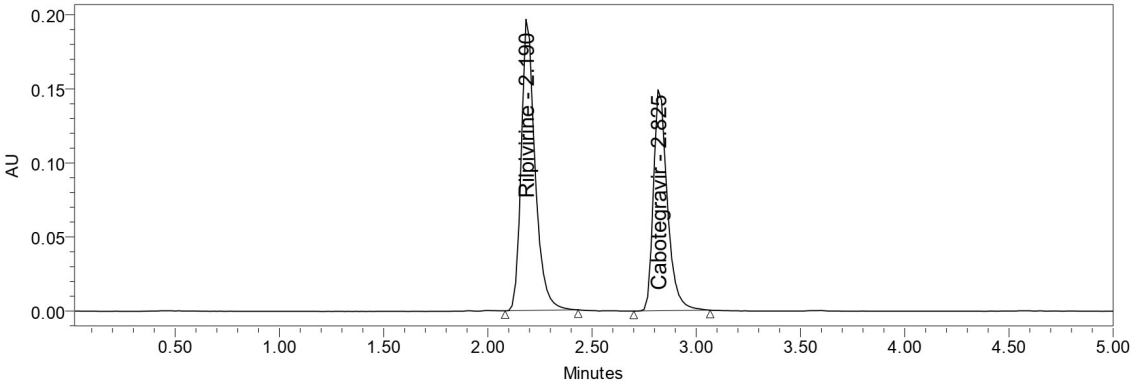

Fig 2: System suitability Chromatogram

DISCUSSIONS

Nephrolithiasis, or kidney stone disease, represents a significant global health concern due to its increasing prevalence and complex multifactorial etiology. Recent epidemiological trends reveal that nearly 1 in 10 individuals will experience a kidney stone event in their lifetime, underscoring the urgent need for improved prevention and management strategies. Factors such as dietary habits, genetic predispositions, and metabolic disorders significantly influence the formation and recurrence of kidney stones. The economic and health burdens of nephrolithiasis are considerable, impacting productivity and necessitating advanced healthcare interventions.
Advancements in medical research have provided valuable insights into the pathogenesis of kidney stones. Understanding the roles of dietary oxalates, sodium, and genetic factors has enabled the development of tailored preventive measures. Diagnostic improvements, including low-dose CT scans, ultrasound, and the use of biomarkers, have enhanced early detection and characterization of stones. Furthermore, minimally invasive surgical techniques like ureteroscopy and laser lithotripsy have revolutionized treatment, offering higher precision, reduced recovery times, and improved patient outcomes.

Innovations in technology, particularly artificial intelligence and machine learning, have shown promise in predicting stone composition and recurrence risk, enabling more personalized care. These methodologies, combined with virtual reality and radiomics, have improved surgical planning and execution, offering safer and more effective outcomes. Preventative strategies emphasize the importance of adequate hydration, dietary modifications, and pharmacological interventions such as potassium citrate to address underlying metabolic abnormalities.

Globally, disparities in nephrolithiasis management remain a challenge, particularly in lower- and middle-income countries, where access to advanced diagnostic tools and minimally invasive treatments is limited. Addressing these inequities requires the implementation of telemedicine, community-based education, and culturally sensitive health programs.

In conclusion, while nephrolithiasis continues to pose significant health challenges, ongoing research and technological advancements are shaping the future of its management. Emphasizing prevention, personalized medicine, and equitable healthcare policies will be critical in reducing the global burden of this condition

CONCLUSION

The developed RP-HPLC method is a reliable, efficient, and cost-effective tool for the simultaneous estimation of Rilpivirine and Cabotegravir in pharmaceutical dosage forms. Its stability-indicating nature ensures its applicability for routine quality control in industrial settings. Retention time of Rilpivirine and Cabotegravir were found to be 2.174 min and 2.815 min. %RSD of the Rilpivirine and Cabotegravir were and found to be 0.4 and 0.8 respectively. %Recovery was obtained as 99.51% and 100.35% for Rilpivirine and Cabotegravir respectively. LOD, LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.38, 1.15 and 0.06, 0.19 respectively. Regression equation of Rilpivirine is y = 15223x + 1240.7 and y = 17119x +

1690 of Cabotegravir Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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