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A comprehensive review on analytical methods of Cabotegravir

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ABSTRACT

HIV weakens the immune system, but highly active antiretroviral therapy (HAART), including the FDA-approved Cabotegravir, has improved life expectancy for those living with HIV. However, these treatments require lifelong use and can have side effects that impact adherence. Reliable methods to detect and measure Cabotegravir in pharmaceutical formulations and biological samples are crucial for ensuring its effectiveness. Chromatographic techniques like High-Performance Liquid Chromatography (HPLC), Reverse-Phase HPLC (RP-HPLC), Ultra-Performance Liquid Chromatography (UPLC), and High-Performance Thin-Layer Chromatography (HPTLC) are widely used for its analysis. These methods vary in sensitivity and efficiency, and choosing the right method depends on the sample type and required detection limits. This review explores these techniques, aiding researchers in selecting the most suitable approach for routine drug testing, enhancing the accuracy and efficiency of antiretroviral drug analysis, and ultimately improving HIV treatment outcomes.

Keywords: AIDS, HIV, Cabotegravir, Method development, validation.

I. INTRODUCTION

Cabotegravir, a potent antiretroviral drug approved by the US FDA in 2021, is a structural analogue of dolutegravir (Fig. 1), belonging to the class of integrase strand transfer inhibitors (INSTIs).[1,2] It exerts its antiviral activity by binding to the active site of HIV integrase, thereby preventing the integration of the viral genome into the host's DNA and halting further replication of the virus. This mechanism effectively reduces viral load, making Cabotegravir a vital component in the treatment and prevention of HIV infections.

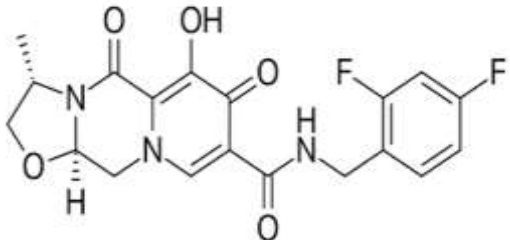

Fig. 1: Structure of cabotegravir

One of the distinguishing features of Cabotegravir is its long duration of action, which offers a significant advantage over conventional antiretroviral therapies.[3] It is available in two dosage forms: an oral tablet taken once daily and a long-acting intramuscular injection administered monthly. The extended dosing interval not only improves patient compliance but also reduces the burden of daily pill intake, making it a more convenient and effective option for individuals with adherence challenges.

Cabotegravir, known for its efficacy, safety, and extended dosing schedule, is widely used for HIV PrEP and treatment.[4] Its growing clinical importance necessitates reliable analytical methods for quantification in pharmaceutical and biological matrices. Various techniques, including UV spectrophotometry and RP-HPLC, have been explored, with RP-HPLC being the most preferred due to its simplicity, accuracy, and sensitivity. Studies focus on both individual and simultaneous drug estimation.[5-8] Given Cabotegravir's expanding use, a comprehensive review of available analytical methods is crucial to identifying the most effective techniques, addressing gaps, and guiding future research. This review critically evaluates current methodologies, highlighting their advantages and limitations to support further development in pharmaceutical analysis.

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Available analytical techniques:

Many analytical techniques were reported for the quantification of cabotegravir either alone or in combination with other antiviral agents. The details were given in the following sections.

UV-Visible Spectrophotometry

A thermosensitive and mucoadhesive vaginal gel was developed to enhance deliveryof cabotegravir.[9] A UV-visible spectrophotometry method was validated in methanol, simulated vaginal fluid (SVF), and vaginal tissue to quantify cabotegravir in gel formulations, in vitro, and ex vivo studies per ICH guidelines. Calibration curves were linear ($R^2 \geq 0.998$), with LLOQ values of 2.15 µg/mL (methanol), 2.22 µg/mL (SVF), and 5.13 µg/mL (tissue). The method was accurate, precise, and suitable for quantifying cabotegravir, ensuring uniform drug content and controlled 24-hour release. Further analytical methods are needed for in vivo quantification.

High performance Liquid Chromatography (HPLC)

A simple, precise, and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Rilpivirine and Cabotegravir in pharmaceutical dosage forms. The separation was achieved on a Sunfire C18 column (150 × 4.6 mm, 5 µm) using a mobile phase of 0.1% orthophosphoric acid and acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min.[10] Retention times were 2.174 min for Rilpivirine and 2.815 min for Cabotegravir. The method showed excellent linearity, accuracy, and precision, with %RSD values below 2%, recovery between 99.51% and 100.35%, and LOD/LOQ values of 0.38/1.15 µg/mL for Rilpivirine and 0.06/0.19 µg/mL for Cabotegravir. This robust and cost-effective stability-indicating method is suitable for routine quality control applications in the pharmaceutical industry.

In a stability-indicating RP-HPLC method, both cabotegravir and rilpivirine were simultaneously estimatedusing anInertsil C18 column (150 × 4.6 mm, 5 µm) and a mobile phase of 0.01N ammonium acetate buffer (pH 3) and acetonitrile (65:35, v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 257 nm.[11] Cabotegravir and rilpivirine showed retention times of 2.250 and 2.823 minutes, respectively. The method was linear ($R^2 = 0.999$) in the ranges of 10–60 µg/mL and 15–90 µg/mL. It was precise, robust (%RSD < 2%), and showed excellent recovery (~100%). The highest degradation was noticed by oxidation.

Simultaneous quantification of cabotegravir and rilpivirine in injection dosage form was achieved on an Agilent BDS C18 column (150 × 4.6 mm, 5 µm) using a mobile phase of 0.01N KH_2PO_4 buffer (pH 4.8): acetonitrile (70:30 v/v) at 260 nm.[12] Retention times were 2.30 min for cabotegravir and 3.187 min for rilpivirine. The method exhibited excellent linearity ($r^2 = 0.9999$) in the ranges of 25–150 µg/mL and 37.5–225 µg/mL for cabotegravir and rilpivirine, respectively. Precision studies showed %RSDs below 0.5%, and recovery was within 99.79%–100.25%. The method was validated for LOD/LOQ (0.24/0.74 µg/mL for cabotegravir, 1.10/3.34 µg/mL for rilpivirine) and successfully applied to forced degradation studies. Significant degradation was observed under acidic, basic, and oxidative conditions, confirming its stability-indicating capability. This method is suitable for routine analysis of these drugs in both bulk and injectable formulations.

In another chromatographic method, cabotegravir and rilpivirine were separated using a Phenomenex Gemini column (250 × 4.6 mm, 5 µm) with methanol:phosphate buffer (pH 4.2) in a 20:80 (v/v) ratio as the mobile phase, at 1.0 mL/min and detection at 246 nm. The method showed linearity for cabotegravir (20–100 µg/mL) and rilpivirine (40–120 µg/mL), with R^2 = 0.999 for both. LOD/LOQ were 0.98/1.27 µg/mL (CAB) and 2.94/3.81 µg/mL (RPV). Mean recoveries ranged from 98–102%, with %RSD within acceptable limits for precision. The method was accurate, precise, specific, and robust, making it suitable for routine quality control.[13]

A reverse-phase HPLC method was developed and optimized for the simultaneous analysis of the anti-HIV drugs cabotegravir (CAB) and rilpivirine (RILP) in pure API, intramuscular injection dosage forms, and human plasma.[14] The method enables rapid analysis using minimal drug quantities. Linearity was established in the concentration ranges of 2.5–15 µg/mL for CAB and 3.75–22.5 µg/mL for RILP, with excellent correlation coefficients (R^2 = 0.999 for both). Chromatographic separation was achieved using a Kinetex C18 column (250 × 4.6 mm, 5 µm) with detection at 242.5 nm. Retention times were 2.14 min for CAB and 3.12 min for RILP. The validated method is suitable for accurate and efficient analysis of CAB and RILP in individual and combined formulations.

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High performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS)

A sensitive and selective HPLC-MS method was developed for simultaneous quantification of rilpivirine (RPV) and cabotegravir (CAB) in various biological matrices, including plasma, skin, lymph nodes, vaginal tissue, liver, kidneys, and spleen of female Sprague Dawley rats. Sample preparation involved a one-step protein precipitation using acetonitrile. Separation was achieved on an Inertsil ODS-3 column using isocratic elution with acetonitrile and 0.1% trifluoroacetic acid in water (81:19, v/v) at 0.3 mL/min, with a 13 minute run time. RPV and CAB were detected at m/z 367.4 and 406.3, respectively. The method was validated according to ICH guidelines, showing accuracy, precision, selectivity, and sensitivity. It was successfully applied to quantify both drugs in in vivo samples for biodistribution studies.[5]

A novel LC-MS/MS assay was developed and validated for quantifying cabotegravir (CAB) and rilpivirine (RPV) in dried blood spots (DBS), offering a practical alternative to plasma-based monitoring.[15] Whole blood was spiked with CAB and RPV, spotted onto DBS cards, extracted, and analyzed using isotopically labeled internal standards. The method showed linearity across 25–20,000 ng/mL for CAB and 2–2500 ng/mL for RPV, with acceptable precision, accuracy, and matrix effects. While RPV remained stable under all storage conditions, CAB exhibited some degradation. DBS concentrations were lower than paired plasma samples by 54.0% (CAB) and 14.1% (RPV). Conversion factors (1.79 for CAB, 1.16 for RPV) were applied to accurately estimate plasma concentrations. These estimates showed strong agreement with measured plasma levels, validating the method's reliability. This approach enhances therapeutic drug monitoring and pharmacokinetic assessment, particularly in large-scale or resource-limited settings.

Ultra-High Performance Liquid Chromatography (UHPLC)

A reversed-phase ultra-high performance liquid chromatography (UHPLC) method was developed for simultaneous quantification of cabotegravir (CAB) and the E-isomer of rilpivirine (RPV) in human EDTA plasma, considering the instability of both drugs under light. The (Z-isomer RPV/total RPV) ratio was measured for all samples to ensure reliability. [$^{2}H_{3}$]-CAB and [$^{13}C_{6}$]-RPV served as internal standards. Sample preparation involved protein precipitation using methanol. Chromatographic separation was achieved using an HSS T3 column (40 °C), with a mobile phase of 65% aqueous formic acid (0.1%) and 35% acetonitrile (0.1%) at 0.5 mL/min. Detection was performed by MS/MS in a 3.0-minute run. The validated assay ranged from 0.0500–10.0 mg/L for CAB and 0.00300–3.00 mg/L for RPV, with within- and between-day accuracy of ~101% (CAB) and ~98% (RPV), and precision (CV) of 5%. The assay has been implemented in therapeutic drug monitoring for HIV patients on CAB/RPV therapy and is suitable for future pharmacokinetic studies.[16]

An ultra-high performance liquid chromatography (UHPLC) method compatible with mass spectrometry was developed for the determination of eight cabotegravir-related impurities, including its degradation products. The method differentiates cabotegravir from its impurities with high specificity. Analytical Quality by Design (AQbD) principles guided the development, ensuring robustness within a defined Method Operable Design Region (MODR): flow rate (0.32–0.40 mL/min), column temperature (30–40 °C), mobile phase A pH (3.25–3.75), and final acetonitrile percentage in the gradient (50–60%). An optimal working condition was selected: flow rate of 0.36 mL/min, column temperature of 35 °C, mobile phase pH of 3.5, and 55% acetonitrile. The method was fully validated, meeting criteria for accuracy, repeatability, linearity, response factors, and detection and quantification limits. All validation parameters met acceptance thresholds, confirming the method's reliability. This robust and sensitive method is suitable for quality control and development of new pharmaceutical products containing cabotegravir.[17]

A multiplex ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) bioassay was developed for the simultaneous quantification of bictegravir, cabotegravir, doravirine, and rilpivirine in plasma from people living with HIV.[18] The method involves simple protein precipitation and direct supernatant injection, achieving rapid separation of all four analytes in under 3 minutes using reversed-phase chromatography and triple quadrupole detection. The assay was fully validated according to international guidelines, demonstrating excellent trueness (94.7%–107.5%), repeatability (2.6%–11%), and intermediate precision (3.0%–11.2%) across clinically relevant concentration ranges. This sensitive and robust bioanalytical method is currently implemented for

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routine therapeutic drug monitoring of oral bictegravir and doravirine, and is intended for future monitoring of long-acting injectable cabotegravir/rilpivirine formulations administered monthly or bimonthly.

II. CONCLUSION

Cabotegravir, a long-acting integrase inhibitor, plays a vital role in HIV treatment and prevention due to its efficacy and improved patient adherence. As its clinical use expands, precise and validated analytical methods are essential for accurate quantification in pharmaceutical formulations and biological matrices. This review highlights various techniques such as UV spectrophotometry, RP-HPLC, HPLC-MS, and LC-MS/MS, emphasizing their sensitivity, specificity, and applicability. Among these, RP-HPLC remains the most widely adopted due to its robustness and cost-effectiveness, while advanced techniques like LC-MS/MS offer high sensitivity for pharmacokinetic studies. Each method serves a specific purpose, depending on the sample type and intended analysis. The availability of these diverse analytical tools strengthens quality control, supports effective therapeutic monitoring, and ultimately enhances the outcomes of HIV treatment involving Cabotegravir.

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