

A Review on Analytical Methodologies for the Estimation of Anti-Retroviral Drugs Cabotegravir and Rilpivirine

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ABSTRACT

Acquired Immune Deficiency syndrome (AIDS) is infections resulting from the damage to the human immune system. A significant shift in the prognosis of individuals living with HIV has occurred due to the widespread use of highly active antiretroviral treatments. Unfortunately, the lifelong side effects of such treatments raise concerns about maintaining therapeutic efficacy as well as long-term tolerability.

The FDA recently approved the combination of the most promising leading molecules, Cabotegravir and Rilpivirine, in 2021. The objective of this manuscript is to critically analyze the existing literature concerning a range of chromatography techniques that can be used concurrently and separately to ascertain the presence of antiretroviral medications in both biological samples and pharmaceutical preparations. These two drugs Cabotegravir and rilpivirine were selected because of their extensive use as anti-viral drug in combination.

The development and validation of such therapeutic agents are challenging work for the analysts. Therefore, the proposed review integrally addresses the analytical reports of Cabotegravir and Rilpivirine recorded in the literature databases like Scopus, Web of Science, Google Scholar, Pub-Med, and through many other sources. It has been remarked that for the development of many analytical techniques were used for addressing the qualitative and quantitative estimation of Cabotegravir and Rilpivirine from various pharmaceutical and biological matrices. The hyphenated and chromatographic techniques are frequently used for analysis of cited drug. The review provides an overview of different chromatographic techniques, such as HPLC, RP-HPLC, UPLC and HPTLC. This will facilitate the analytical chemist to make use of the method for the routine analysis for the antiviral drug Cabotegravir and Rilpivirine. It will also focus on several available chromatographic conditions with different instruments used. So it will reduce time for the analytical chemist required for the analytical method development and also helps in formulation development.

Keywords: AIDS, HIV, Cabotegravir, Rilpivirine, Method, Development, Validation.

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LIST OF ABBREVIATIONS

HIV: Human Immunodeficiency Virus; FDA: Food and Drug Administration; HPLC: High-Performance Liquid-Chromatography; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; UHPLC: Ultra-High Performance Liquid Chromatography; HPTLC: High-Performance Thin-Layer Chromatography.

INTRODUCTION

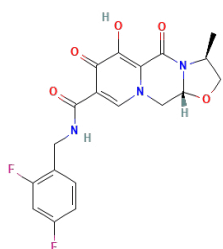
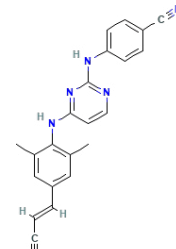
There are around 39.0 million cases of Human Immunodeficiency virus (HIV) recorded in whole world till 2022 by WHO [1]. Lacking of immune system, that normally act as shield against viral attack, manifests by occurrence of repeated viral infection in particulate having immune deficiency disease. There are two main strains or types of HIV: HIV-1 and HIV-2. HIV act by weakening the immune system by infecting and destroying CD4+ T cells, which further leads to immunodeficiency at later stage of disease. The use of potent antiretroviral therapy (ART) in patients infected with HIV (Type-1) reduces morbidity and mortality and often results in substantial recovery of impaired immunologic function [2]. Already discovered Anti-Retroviral Therapy (ART) is highly effective — almost completely suppressing HIV replication, improving immune function and significantly reducing the risk of developing AIDS. However, ART is not curative; if drugs are

stopped, the virus nearly invariably rebounds within weeks [3]. The recent approval of a combined treatment regimen utilising prolonged-release suspensions of the antiretroviral agents cabotegravir (CAB) [4] and Rilpivirine (RPV) [5] for monthly intramuscular injections was a great step forward towards improved HIV treatment regimens.

Cabotegravir is an Integrase inhibitor which is chemically (N-((2,4-Difluorophenyl) methyl)-6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a-hexahydro (1,3) oxazolo (3,2-a) pyrido (1,2-d) pyrazine-8- carboxamide. It is structural analogue of dolutegravir. Cabotegravir attaches to the active site of HIV integrase, preventing genetic material transfer of the viral genome into the host genome, and preventing duplication of the virus [6]. It is available as tablets and as intramuscular injection.

Rilpivirine is a second-generation non-nucleotide reverse transcriptase inhibitor (NNRTI) which is chemically 4-[[4-[(E)-2- cyanoethenyl] - 2, 6-dimethyl-anilino] pyrimidin-2-yl] amino] benzonitrile [7]. Rilpivirine is also known as Edurant and Rekambys [8]. It is a diaryl pyrimidine derivative [9]. It's binding results in the blockage of RNA and DNA- dependent DNA polymerase activities [10]. It is available in injectable form. Physical and chemical properties of both Cabotegravir (CAB) and Rilpivirine (RIL) are discussed in table (1).

Table 1. Physical and chemical properties of CAB and RIL [11-18]

Title	Cabotegravir	Rilpivirine
Structure		
Mol. Formula	C ₁₉ H ₁₇ F ₂ N ₃ O ₅	C ₂₂ H ₁₈ N ₆
Mol. Weight	405.4 g/mol	366.4 g/mol
Melting point	248-251°C	241-243°C
CAS number	1051375-10-0	500287-72-9
Log P	1.04	4.32
pKa	10.04	3.26
λ _{max}	226, 248, 258nm	280nm

Intramuscular administration of drugs permits absorption into the systemic bloodstream, bypassing first-pass metabolism. After getting absorbed, mixed in large volumes of distribution and shows high degree of protein binding for CAB and RPV (>99.8% and 99.7%, respectively). Cabotegravir metabolised through the enzyme UGT1A1 and similarly rilpivirine is metabolised by CYP3A. The main route for cabotegravir elimination is hepatic metabolism [19]. The elimination half-lives for CAB and RIL when administered intramuscularly are 5.6-11.5 weeks and 13-28 weeks, respectively [20].

The trial indicated that oral cabotegravir/rilpivirine was non-inferior in HIV-positive individuals who had not taken any previous treatment [21]. In phase III trials, observed that switching to intramuscular cabotegravir/ rilpivirine was non-inferior to oral dolutegravir/ abacavir/ lamivudine in virally suppressed HIV-positive patients [22].

The most common adverse effect observed with long-acting cabotegravir was injection site reactions, besides headache and pyrexia. In the ATLAS study, the following adverse effects associated with the trial regimen were identified: injection site reaction (64%), pyrexia (4%), nausea (1%), diarrhoea (1%), headache (4%), and lipase rise (1%) [23]. Cabotegravir and rilpivirine have a low potential to cause Drug-drug interactions (DDI). There is no evidence that cabotegravir and rilpivirine produce clinically significant DDI through protein displacement. Rilpivirine has a higher potential for DDIs as compared with cabotegravir which is explained by the higher risk of QT interval prolongation associated with Rilpivirine. Rilpivirine should be used cautiously, especially when coupled with medications known to increase the risk. In contrast, cabotegravir did not affect cardiac repolarisation when given in super therapeutic dose [19].

ANALYTICAL STATUSES ON CAB & RIL

In the pharmaceutical field, it is very significant to analyse bulk drug materials; drug ingredients, drug intermediates, impurities, marketed formulations, and degradation products. Since CAB & RIL were recently approved, there are still lots of unexplored studies. However, from the literature reports that are available, numerous useful analytical tools, including UV-spectroscopy, HPLC (high performance liquid chromatography), RP-HPLC (reverse phase-high performance liquid chromatography), HPTLC (high performance thin layer chromatography), LC-MS (liquid chromatography-mass

spectroscopy), UHPLC-MS/MS (ultra-high performance liquid chromatography-mass spectroscopy), etc. are studied. These analytical techniques are commonly used and reliable for the analytical chemist for the routine analysis. After extensive literature survey, several validated methods were found in the different databases. So this review article aims to collect and summarise the different chromatographic conditions which will be beneficial for the analytical chemist. This review article also helps to select chromatographic conditions for the routine analysis of this widely used combination.

AVAILABLE ANALYTICAL TECHNIQUES

High performance liquid chromatography

HPLC is widely used mainly for two reasons, one is for qualitative analysis of unknown mixtures and one is for mixtures separation for the later analysis. All HPLC methods were precisely summarised in table 2. Inertsil ODS-3 column (4.6 mm × 250 mm, 5 µm particle size) with a solvent system consisting of acetonitrile and 0.1 percent (v/v) trifluoroacetic acid in water (81:19, v/v) was used for the analysis of Separation of the CAB and RIL in rat plasma tissues was monitored at 257 nm. The flow rate was kept at 0.3ml/min for run time of 13 min. The values of the correlation coefficient (R₂) varied from 0.9998 to 1.00. It was discovered that the limits of quantification (LOQ) is 5ng/ml for both and limit of detection (LOD) were, 4.77ng/ml and 9.93ng/ml for rilpivirine and cabotegravir respectively [24]. Another method for measuring rilpivirine and cabotegravir in pharmaceutical active ingredients. A symmetry C18 column (150 x 4.6 mm, 3.5) with developmental phase of 0.1 percent formic acid and acetonitrile (80:20) was used to study the separation of the same from the matrix using isocratic elution at 231 nm. The results of the CAB and RIL analyses showed excellent linear responses for the proposed work at concentrations of 30–450 µg/mL and 20–300 µg/mL, respectively. For Rilpivirine and Cabotegravir, the corresponding LOD and LOQ were 0.375 µg/mL, 1.238 µg/mL, and 0.25 µg/mL, 0.825 µg/mL, respectively [25].

RP-HPLC method was developed for the Simultaneous Estimation of Cabotegravir and Rilpivirine in the bulk and pharmaceutical dosage form with UV detection at 275 nm. The calibration curve was linear over the concentration range of 10-60 µg/ml and 15-90 µg/ml for CAB and RIL, respectively. The chromatographic separation was performed

at 30°C temperatures on an inertsil C18 (150 x 4.6 mm, 5 µm) column analytical with a mobile phase consisting of 0.01N ammonium acetate buffer (pH 3) and acetonitrile (65:35, v/v). Flow-rate was 0.1 mL/min. Detection was measured at 275 nm. Retention times of CAB, and RIL were 2.250 min and 2.823 min, respectively. For cabotegravir, the LOD and LOQ were found to be 0.13µg/ml and 0.38µg/ml, respectively, and for rilpivirine, 0.16µg/ml and 0.48µg/ml, respectively. Total run time was 5 minutes. Additionally, correlation coefficient (R²) of 0.999[25].

Another method for estimating Cabotegravir and Rilpivirine in bulk drugs and marketed pharmaceutical dosage form using Reverse phase high performance liquid chromatography (RP-HPLC). The highest performance parameters were achieved by optimizing the chromatographic conditions using Phenomenex Gemini (250mm x 4.6mm) 5µm Particle size Column with guard filter at 32°C. The separation was carried out using a mobile phase containing Methanol and Phosphate Buffer pH-4.2 in the ratio of 20: 80 v/v pumped at a flow rate of 1.0 mL/min for the run time of 7 min. Detection was performed using PDA detector at 246 nm. The method demonstrated linearity in the concentration range of 20–100 µg/mL and 40–120µg/mL for cabotegravir and rilpivirine, respectively, with regression coefficients of 0.999 for both. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.98µg/mL and 1.27µg/mL & 2.94µg/mL and 3.81µg/mL for Cabotegravir and Rilpivirine, respectively [26].

The development and validation of a stability method for the anti-HIV medications cabotegravir and rilpivirine was reported for intramuscular injection. A separation of the same from the human plasma matrix was examined at 25°C using a Kinetex C-18 column and Hypersil ODS (250 mm x 4.6 mm id, 5 µ) column. Acetonitrile (ACN) (35:65) Sodium Dihydrogen Phosphate buffer (0.05M) of pH 5.5. as mobile phase at 242.5 nm. The analysis results of CAB & RIL showed excellent linear response for CAB 2.5 to 15 g/ml and for RIL 3.75 to 22.5 g/ml, and R² of both drugs was found to be 0.9999 for the suggested work. The LOD and for Rilpivirine and Cabotegravir were 0.263 µg/mL, 0.202 µg/mL, respectively and similarly LOQ were 0.798 µg/ml and 0.613 µg/ml [27].

The development and validation of a stability method for the anti-HIV medications cabotegravir and rilpivirine was conducted a study on the determination of cabotegravir

and rilpivirine in a pharmaceutical dosage form using the RP-HPLC method. The analysis involved the separation of cabotegravir and rilpivirine from pharmaceutical dosages. The chromatogram obtained by using a Kromasil C18 column with dimensions of 150 x 4.6 mm and a particle size of 5µ. A mobile phase consisting of a mixture of Buffer 0.01N Potassium dihydrogen phosphate and Acetonitrile in a ratio of 60:40 was used by through a column at a flow rate of 1.0 ml/min. The temperature maintained at 30°C. The optimized wavelength chosen for analysis was 257 nm. The retention times of cabotegravir and Rilpivirine were determined to be 2.642 min and 2.257 min, respectively. Linearity was established at Six linear concentration of Cabotegravir and Rilpivirine was 18.75-112.5µg/ml and 12.5- 75µg/ml. From the regression equations for cabotegravir and rilpivirine, the LOD and LOQ values were, respectively, 0.18, 0.54, and 0.15, 0.46µg/ml. Another study conducted in which they employed the RP-HPLC method to determine the amount of rilpivirine present in a pharmaceutical dosage form. The separation and analysis of rilpivirine from the pharmaceutical dosages were accomplished using an Agilent Zorbax Bonus-RP column measuring 250 x 4.6 mm, with a particle size of 5µ. The mobile phase consisted of a mixture of Sodium Dihydrogen Phosphate (Buffer) and acetonitrile (20:80). The flow rate of the mobile phase was maintained at 1 mL/min, while the detection wavelength was 304 nm. The minimum concentration of rilpivirine that could be detected (LOD) was determined to be 2.35µg/ml, whereas the minimum concentration that could be quantified (LOQ) was found to be 7.13 µg/ml [28]. A reversed phase high performance liquid chromatographic method was developed to ascertain the injection dosage form of rilpivirine and cabotegravir. A 0.01N KH₂PO₄ buffer (pH: 4.8) and acetonitrile (70:30v/v) mobile phase were used in the analysis, which was conducted on an Agilent - C18 column (BDS) measuring 150 X 4.6 mm and particle size 5µ at 260 nm wavelength. Retention durations of 2.30 and 3.187 minutes respectively, The method's linearity between 25 and 150 µg/mL for cabotegravir and 37 to 25 µg/mL for rilpivirine. It was discovered that both medications had a correlation coefficient (R²) of 0.9999. A photodiode-array detector-based RP-HPLC technique was described for the estimation of dolutegravir and rilpivirine in bulk pharmaceutical dosage form and rat plasma. Using an isocratic elution mobile phase made up of acetonitrile and buffer (0.1 percent o-phthaldialdehyde) in a 60:40 v/v ratio, chromatographic separation was carried out

on a Phenomenex C18 (150x4.6mm, 5 μ m) column. During a 10-minute run, the flow rate was maintained at 1.0 millilitres per minute. At 262nm, detection was observed. 7.73 minutes were found to be the retention times. About 0.005 μ g/ml was the LOD. The LOQ value was approximately 0.05 μ g/ml for rilpivirine. In the concentration range of 5–75 μ g/ml for rilpivirine, the suggested method exhibits good linearity [29]. A study conducted which utilized a photodiode-array detector to simultaneously determine the concentrations of rilpivirine and dolutegravir in a bulk dosage formulation. This was achieved using a reversed-phase high-performance liquid chromatography (RP-HPLC) method. The chromatographic separation was performed on an Agilent C18 column with dimensions of 4.6 x 150mm and a particle size of 5 μ m. The mobile phase consisted of a mixture of 0.1% Ortho phosphoric acid buffer and acetonitrile in a ratio of 50:50 v/v. The flow rate was maintained at 1 ml/min throughout the 5-minute run time. The temperature of the system was set at 30°C. The optimized wavelength for detection was 257 nm. The retention time for rilpivirine was determined to be 2.859 min. The limit of detection (LOD) and limit of quantification (LOQ) for rilpivirine were found to be approximately 0.56 μ g/ml and 1.69 μ g/ml, respectively. The proposed method exhibited good linearity in the concentration range of 6.25–37.25 μ g/ml for Rilpivirine [30]. According to a Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Emtricitabine, Rilpivirine, Tenofovir Disoproxil Fumarate, and their pharmaceutical dosage forms. The chromatographic separation was achieved using gradient elution techniques on an Inertsil ODS 3V column with dimensions of 150 mm x 4.6 mm and a particle size of 5 μ m. The method employed two mobile phases, the first being 0.02M Sodium Dihydrogen Orthophosphate Monohydrate, and the second consisting of a mixture of methanol and water in a ratio of 85:15 v/v. The flow rate was set at 1.5 mL/min, and the total run time was 15 minutes. The detection wavelength was monitored at 270 nm. The retention time for Rilpivirine was found to be 12.020 minutes. The linear range of detector response for Rilpivirine was determined to be 20–360 μ g/ml [31]. One more method developed by using RP-HPLC for the simultaneous estimation of tenofovir disoproxil fumarate, emtricitabine, and rilpivirine hydrochloride in pharmaceutical dosage forms as nano suspension. The chromatographic separation was carried out on BDS hypersil C18 (4.6 x 250mm, 3 μ m) HPLC column

with a mobile phase consisting of methanol, water, and acetonitrile (80:13.4:6.6) V/V at a flow rate of 1.0mL/min. 306 nm was the ideal wavelength for detection. It was found that rilpivirine had a retention time of 31.683 minutes. It was discovered that the limits of quantification (LOQ) and detection (LOD) for rilpivirine were roughly 0.613 μ g/ml and 0.202 μ g/ml, respectively. For rilpivirine, the suggested method demonstrated good linearity in the concentration range of 0.625–7.5 μ g/mL [32].

Ultra-high performance liquid chromatography

Ultra-High Performance Liquid Chromatography (UHPLC) has recently been introduced; provide new opportunities for the development and evaluation of methods. Liquid chromatography with ultra-high performance (UHPLC) involves LC separations with columns that have particles smaller than the 2–5 μ m sizes commonly used in HPLC. Greater efficiency per unit time and increased linear velocities are the advantages of using columns containing smaller particles, usually smaller than 2 μ m [33]. Based on our research, UHPLC can reduce run times by up to 7 times when compared to traditional HPLC [34]. All UHPLC methods were precisely summarised in table 3.

An ultra-high performance liquid chromatographic method was developed to simultaneously quantify cabotegravir and rilpivirine E-isomer in human plasma. The analysis utilized an HSS T3 column with dimensions of 100 x 2.1 mm and 1.8 μ m particle size, with a mobile phase consisting of 65% 0.1% formic acid in water (A) and 35% 0.1% formic acid in acetonitrile (B) at a flow rate of 0.5 mL/min. Detection was achieved through tandem mass spectrometry (MS/MS) with a total runtime of 3.0 minutes. During validation, the regression coefficients (R²) for all three calibration curves were 0.9989 \pm 0.0008 and 0.9995 \pm 0.0006 for CAB and RPV E-isomer in EDTA-plasma, respectively. The LLOQ and HLOQ for CAB were 0.05005 and 10.01mg/mL, while the LLOQ and HLOQ for RIL were 0.003001 and 13.001 mg/mL [35]. A study conducted which introduced a reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) method for the simultaneous quantification of (E) and (Z) isomers of rilpivirine and four degradation products in bulk and tablets. The analysis utilized an Acquity ethylene bridged hybrid Shield RP18 column with dimensions of 150 mm x 2.1 mm and a particle size of 1.7 μ m. The temperature of the column was maintained at 35 °C, and the mobile phase consisted

of mixture of acetonitrile and 0.05% formic acid in 10 mM ammonium formate. The detection wavelength employed for this study was 305 nm, and the flow rate of the mobile phase was set at 0.3 mL/min for a run time of 13 min. To evaluate the linearity of the developed method, a concentration range of 0.050–150.0 µg/mL for cabotegravir was examined. The limit of detection (LOD) and limit of quantification (LOQ) values for rilpivirine were found to be 0.03 µg/mL and 0.05–150 µg/mL, respectively. The obtained correlation coefficient (R₂) was determined to be 0.9999[36]. A method utilizing ultra-high performance liquid chromatography (UHPLC) was established for the purpose of determining the related substances and degradation products of Cabotegravir. The analysis was carried out using an Acquity UPLC BEH Phenyl column, with dimensions of 150 mm × 2.1 mm and a particle size of 1.7 µm. The column temperature was maintained at 35 °C, and the mobile phase consisted of two components: A, which was a mixture of 0.5% formic acid and acetonitrile (94:6, v/v), and B, which was a combination of methanol and acetonitrile (94:6, v/v). The detection wavelength employed for this study was 258 nm, and the flow rate of the mobile phase was set at 0.3 mL/min. The linearity of the developed method was evaluated over a concentration range of 0.05µg/mL to 0.20µg/mL for cabotegravir. The LOD and LOQ values were found to be 0.125µg/mL and 0.25µg/mL, respectively for CAB. The obtained correlation coefficient (R₂) was determined to be 0.999[37]. An ultra-high performance liquid chromatographic method developed for the determination of stable isotopic internal standards for the monitoring of plasma concentrations of the antiretroviral medications bictegravir, cabotegravir, doravirine, and rilpivirine in people. The analysis was conducted using an Ultimate Xselect HSS T3 analytical column with dimensions of 2.1 x 75 mm, 3.5-µm particle size, and mobile phase made up of, respectively, H₂O + 0.1 percent Formic acid (FA) (A) and acetonitrile(ACN) + 0.1 percent FA (B) the multistep gradient at a flow rate of 300 µL/min: a linear gradient from 40 percent to 60 percent B in three minutes, up to 95 percent B in zero minutes, and then an isocratic stage lasting eight minutes at 95 percent B. After that, solvent B was reduced to 40% (the initial conditions) in 0–1 minutes. This was followed by a re-equilibration step that took a total of 5 minutes to complete. R₂, the correlation coefficient, was discovered to be significantly higher than 0.99. The LLOQ for the CAB and RIL were 25 to 10000 ng/mL and 10 to 2000 ng/ml [38]. The UPLC-PDA (stability

indicating) developed for the simultaneous quantitative determination on Multi drug Combination of Emtricitabine, Tenofovir Alafenamide and Rilpivirine in Bulk drug & its tablet formulation. The chromatographic separation was achieved on a Thermosil Octa Decyl (4.6 x 50mm; particle size 1.7 mm) with mobile phase consisting of 35% of 0.1M tri ethyl amine buffer add 65% Acetonitrile HPLC Grade adjusted to pH 3 with Ortho phosphoric acid at a flow rate of 0.3 milliliters per min. at ambient temperature. UV detection was monitored at a wavelength of 215 nm for the PIs and 260 nm for rilpivirine. The runtime was 3.0min [39].

High performance thin layer chromatography

High performance thin layer chromatography (HPTLC) is an advanced technique in planar chromatography. TLC is based on a multi-step distribution process that includes the sample molecules, solvents or solvent mixtures (the mobile phase or eluent), and an appropriate “adsorbent” (the stationary phase) [40]. Compared to classical TLC plates, HPTLC plates are smaller (10 × 10 or 10 × 20 cm), have a thinner (0.1–0.2 mm) layer made of sorbent with a finer mean particle size (5–6 µm) and a narrower particle size distribution or classification (4–8 µm), and are developed over shorter distances (about 3–7 cm). HPTLC plates offer enhanced in situ quantification, reduced analysis time, increased detection sensitivity, and better resolution. Included are HPTLC techniques used in pharmaceutical analysis at various levels, including assays, degradation studies, quantitative impurity determination, qualitative identity testing, and semi quantitative limit tests [41]. HPTLC is a concept that includes both the use of validated techniques for both qualitative and quantitative analysis and a widely standardized methodology founded on scientific facts [42]. All HPTLC methods were precisely summarised in table 4.

To investigate the estimation of Emtricitabine, Rilpivirine, and Tenofovir disoproxil fumarate in a combined dosage form, a study was conducted by using HPTLC. The stationary phase consisted of aluminium plates precoated with silica gel 60 F254, while the solvent system utilized was a mixture of chloroform, ethyl acetate, methanol, and glacial acetic acid in a volumetric ratio of 5:2:1:0.1v/v/v/v. Quantification was performed at a wavelength of 272 nm. The retention factor (R_f) for rilpivirine was determined to be 0.70 ± 0.02. The limit of detection (LOD) and limit of quantification (LOQ) for the same were found to be 4.2500 ng/band and

12.8790 ng/band, respectively. Additionally, linearity for the rilpivirine was observed within the range of 0-300 ng/band [43]. Another method was estimated the combination of Rilpivirine and Dolutegravir sodium in a combined dosage form by HPTLC method. As the stationary phase, the investigation used aluminium plates that had been pre-coated

with silica gel 60 F254. ethyl acetate: methanol: chloroform (8:1:1%v/v/v) were combined in the mobile phase. 254nm was the wavelength at which the compounds were measured. It was found that the retention factor (Rf) was 0.33 ± 0.02 . In addition, the method's linearity was noted in the 500–3000 ng/band range [44].

Table 2. Summary of HPLC Methods

Sr. no.	Chromatographic condition		Validation parameters					References
	Column	Eluent	Detector	Flow rate	Linearity	LOD	LOQ	
1	Co. Apex Scientific Inertsil ODS-3 column (4.6 mm × 250 mm, 5 μm)	Acetonitrile & 0.1% (v/v) trifluoroacetic acid in water (81:19, v/v)	A diode array detector at 257nm	0.3ml/min	-	4.77ng/ml(RIL) 9.93ng/ml (CAB)	5ng/ml	[24]
2	Symmetry C18 column (150 x 4.6 mm, 3.5 μm)	0.1% formic acid & acetonitrile (80:20)	Photodiode array detector at 231nm	1.0 ml/min	(CAB) 20–300 g/MI (RIL)	0.375 g/MI (RIL) 1.238 g/MI (CAB)	0.25 g/MI (RIL) 0.825 g/MI (CAB)	[8]
3	Inertsil C18 (150 x 4.6 mm, 5 μm)	0.01N ammonium acetate buffer (pH 3) and acetonitrile (65:35, v/v)	Photodiode array detector at 275 nm.	0.1 mL/min	10-60 μg/ml(CAB) and 15-90 μg/ml(RIL) 20-100μg/ml	0.13 μg/ml (CAB) and 0.38 μg/ml (RIL)	0.16 μg/ml (CAB)and 0.48 μg/ml (RIL)	[25]
4	Phenomenex Gemini (250mmx4.6mm, 5μm)	Methanol and Phosphate Buffer pH-4.2 in the ratio of 20: 80v/v	PDA detector at 246 nm	0.1 mL/min	(CAB) and 40-120μg/ml (RIL)	0.98 μg/ml(CAB) and 1.27μg/ml (RIL)	2.94μg/mL (CAB)and 3.81μg/mL (RIL)	[26]
5	Kinetex C-18 column and Hypersil ODS (250 mm x 4point 6 mm, 5 m id)	Acetonitrile (ACN) (35) : (65) Sodium Dihydrogen Phosphate buffer (0.05M)	242.5 nm	1ml/min	2.5 to 15 g/ml(CAB) 3.75 to 22.5 g/ml(RIL)	0.263μg/MI (RIL) 0.202 μg/mL (CAB)	0.798 μg/ml (RIL) 0.613 μg/ml (CAB)	[27]
6	Kromasil C18 column (150 x 4.6 mm, 5μ)	0.01N Potassium dihydrogen phosphate and Acetonitrile (60:40)	photodiode array detector at 257nm	1.0 ml/min	18.75-112.5μg/ml (CAB)& 12.5-75μg/ml (RIL)	0.18(CAB) & 0.54 (RIL)	0.15(CAB)& 0.46 (RIL)	[6]
7	Agilent Zorbax Bonus-RP column (250 x 4.6mm, 5μm)	Sodium Dihydrogen Phosphate (Buffer) and acetonitrile (20:80)	diode array detector at 304nm	1.0 ml/min	-	2.35μg/ml (RIL)	7.13 μg/ml (RIL)	[28]
8	Agilent - C18 column (150X 4.6 mm, 5μ)	0.01N KH2PO4 buffer (pH: 4.8) and acetonitrile (70:30v/v)	photodiode array detector at 260nm	1.0 ml/min	25 and 150 μg/ml (CAB) & 37 to 25 μg/ml(RIL)	-	-	[7]
9	Phenomenex C18 (150x4.6mm, 5μm) column	acetonitrile and buffer (0.1% o-phthaldialdehyde) in a 60:40 v/v	photodiode array detector at 262nm	1.0 ml/min	5–75 μg/ml (RIL)	0.005 μg/ml (RIL)	0.05 μg/ml (RIL)	[29]

10	Agilent C18 column (4.6 x 150mm, 5µm)	0.1% Ortho phosphoric acid buffer and acetonitrile(50:50 v/v)	diode array detector at 257nm	1.0 ml/min	6.25-37.25 µg/ml (RIL)	0.56µg/ml (RIL)	1.69µg/ml (RIL)	[30]
11	Inertsil ODS 3V column (150 mm x 4.6 mm,5µm)	0.02M Sodium Dihydrogen Orthophosphate Monohydrate (A) and 0.02M Sodium Dihydrogen Orthophosphate Monohydrate (B)	PDA detector at 270nm	1.5 mL/min	20-360 µg/ml	-	-	[31]
12	BDS hypersilC18 (4.6 x 250mm, 3µm)	methanol, water, and acetonitrile (80:13.4:6.6) V/V	photo diode array detector (PDA) at 306nm	1.0mL/min	0.625-7.5µg/MI (RIL)	0.202µg/ml (RIL)	0.613µg/ml (RIL)	[32]

Table 3. Summary of UPLC Methods

Sr. no.	Chromatographic condition		Validation parameters					References
	Column	Eluent	Detector	Flow rate	Linearity	LOD	LOQ	
1	HSS T3 column (100 x 2.1 mm, 1.8 µm)	65% 0.1% formic acid in water (A) and 35% 0.1% formic acid in acetonitrile (B)	tandem mass spectrometry (MS/MS)	0.5 mL/min	-	-	0.05005 to 10.01mg/mL(CAB) 0.003001 and 13.001 mg/MI(RIL)	[35]
2	Acquity ethylene bridged hybrid Shield RP18 (150 x 2.1 mm, 1.7 µm)	acetonitrile and 0.05% formic acid in 10 mM ammonium formate	a PDA detector at 305nm	0.3 mL/min	0.050-150.0µg/mL (RIL)	0.03µg/mL (RIL)	0.05 - 150µg/mL (RIL)	[36]
3	Acquity UPLC BEH Phenyl column (150 mm x 2.1mm,1.7µm)	0.5% formic acid and acetonitrile (94:6, v/v)(A) A and methanol and acetonitrile (94:6, v/v)(B)	(PDA) or tunable ultraviolet (TUV) optical detector at 258nm	0.3 mL/min	0.05 µg/mL to 0.20 µg/mL(CAB)	0.125 µg/mL (CAB)	0.25µg/mL (CAB)	[37]
4	Ultimate Xselect HSS T3 analytical column(2.1 x 75 mm, 3.5-µm)	H2O + 0.1% FA (A) and ACN + 0.1% FA (B)	MS detector	300 µL/min	-	-	25 to 10 000 ng/mL(CAB)and 10 to 2000 ng/mL(RIL)	[38]
5	Thermosil Octa Decyl (4.6 x 50mm,1.7 mm)	35% of 0.1M tri ethyl amine buffer add 65% Acetonitrile	PDA detector at 215nm	0.3ml/min	-	-	-	[39]

Table 4. Summary of HPTLC Methods

Sr. no.	Chromatographic condition			Validation parameters			References
	Adsorbent	eluent	detector	Rf values	Linearity	LOD	
1	aluminium plates precoated with silica gel 60 F254	chloroform, ethyl acetate, methanol, and glacial acetic acid(5:2:1:0.1v/v/v/v)	at 272nm	0.70 ± 0.02	0-300 ng/band (RIL)	4.2500 ng/band (RIL)	12.8790 ng/band (RIL) [43]
2	aluminium plates precoated with silica gel 60 F254	ethyl acetate: methanol: chloroform (8:1:1%v/v/v)	at 254nm	0.33 ± 0.02	500-3000 ng/band (RIL)	-	- [44]

CONCLUSION

Cabotegravir and Rilpivirine is the most promising leading molecules approved in combination recently 2021 by FDA under brand name cabenuva. Various analytical tools are explained in this review article for CAB & RIL in different pharmaceutical dosage form and biological specimens. For the scientist and researchers in academics and industry, this review could provide a foundation for exploring the development of analytical approaches for the CAB & RIL. In this article, we addressed more than 20 research articles consisting of various chromatographic methods. An overview of the different methods available for measuring the levels of Rilpivirine and Cabotegravir in biological fluids and pharmaceutical formulations is given in this review. RP-HPLC methods display enough ease of use and selectivity towards quantifying cabotegravir and rilpivirine in pharmaceutical formulations and biological fluids. So this review article will be focusing more on the innovation, applicability, and future development trends of analytical methods.

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