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Research Article

DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY ANALYTICAL METHOD FOR THE CONCURRENT QUANTIFICATION OF CABOTEGRAVIR AND RILPIVIRINE IN RAT PLASMA

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ABSTRACT

Objective: To establish and authenticate a precise and dependable liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique for the concurrent quantification of Cabotegravir (CAB) and Rilpivirine (RIL) in rat plasma, specifically for pharmacokinetic studies.

Methods: A protein precipitation extraction procedure was employed, followed by chromatographic separation on a Kromasil C18 column using ethanol and 0.1% triethylamine buffer (30:70, v/v, pH 2.5) as the mobile phase. Quantification was performed using an electrospray ionization interface coupled with a SCIEX QTRAP 5500 mass spectrometer in multiple reaction monitoring mode. The mass transitions for CAB (m/z 406.1240 → 218.3257), RIL (m/z 367.2103 → 174.1127), and the internal standard Raltegravir (m/z 445.1147 → 389.5261) were optimized for maximum sensitivity.

Results: Witnessed excellent linearity ($r^2 > 0.999$), precision (≤15% CV), and accuracy (85–115%) for both intra- and inter-day assays. Recovery rates for CAB and RIL ranged from 94% to 98%. The method exhibited negligible matrix effects, with lower limit of quantitation of 15 ng/mL for CAB and 10 ng/mL for RIL.

Conclusion: This approach provides a robust and sensitive approach for the concurrent quantification of CAB and RIL in rat plasma, suitable for pharmacokinetic studies, offering a reliable tool for future clinical and pre-clinical research on these drugs.

Keywords: LC-MS/MS, Cabotegravir, Rilpivirine, Pharmacokinetics, Bioanalytical validation.

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INTRODUCTION

Cabotegravir (CAB) and Rilpivirine (RIL) are two significant antiretroviral drugs used in HIV therapy. CAB inhibits viral DNA integration, while RIL blocks HIV RNA reverse transcription. When used together, CAB and RIL provide a potent combination for managing HIV, particularly for patients requiring long-acting treatment options [1].

CAB, recently approved for use in extended-release formulations, offers a potential alternative to daily oral therapy by enabling injection-based administration. This innovation could significantly improve patient adherence, particularly for those who struggle with daily pill regimens. Similarly, RIL is primarily used in combination with other antiretrovirals and has shown remarkable action in lowering virus level. Despite the individual importance of CAB and RIL in HIV therapy, there is limited research on their simultaneous quantification in biological samples, specifically rat plasma. Such a method is crucial for understanding their pharmacokinetic profiles and optimizing therapeutic strategies [2].

Pharmacokinetic studies are essential for determining optimal dosing regimens and ensuring that drugs maintain effective therapeutic concentrations in the bloodstream. For such studies, sensitive and reliable approaches are necessary to accurately measure the low concentrations of drugs in plasma samples [3-5].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the benchmark in bioanalysis because of its exceptional sensitivity, specificity, and capability to simultaneously quantify several compounds. It is especially useful for analyzing complex biological matrices, such as plasma, distinguishing drugs from endogenous components. The available literature highlights various valuable analytical techniques, such as ultraviolet [6], High-performance liquid chromatography (HPLC) [7-15], Ultra-performance liquid chromatography [16], Stability indicating [17-19], AQbD [20], LC-MS/MS [21-26], and HPTLC [27,28]. The details of the analytical methods were discussed presented by [29]. To date, no method has been established for the concurrent analysis of CAB and RIL in rat plasma. We aim to establish and validate a reliable, and reproducible methodology for the concurrent quantification of CAB and RIL in rat plasma. This method will serve as a reliable analytical tool for pharmacokinetic studies, enabling researchers to assess the combined pharmacokinetic profiles of CAB and RIL in HIV treatment regimens and to monitor plasma levels during various stages of the treatment process. Furthermore, the method will contribute to the broader field of bioanalysis by providing a well-validated protocol for simultaneous quantification of two drugs in complex biosystems.

METHODS

Chemicals and reagents

CAB and RIL with purity ≥98% were obtained from Cipla Pharmaceutical Ltd., Mumbai, India. The internal standard (IS), Raltegravir, was sourced from CSIR-CDRI, Lucknow, India.

Reagents

Sisco Research Laboratories, Mumbai, India, provided LC-MS grade acetonitrile and methanol while HPLC-grade water is of Milli-Q system

(Millipore, USA). Merck, Mumbai, India provided Triethylamine and formic acid (AR grade).

Chromatographic conditions

Analytes achieved resolution on Kromasil C18 column (150 mm × 4.6 mm, 3.5 µm) (Kromasil, Sweden) with a 30:70 v/v mixture of ethanol and 0.1% triethylamine buffer (pH 2.5), adjusted with formic acid at 1.0 mL/min with a 10 µL injection volume, at ambient temperature, and the run time was 10 min. The system consisted of a Waters e-alliance 2695 HPLC (Waters Corporation, USA), coupled to a SCIEX QTRAP 5500 mass spectrometer (SCIEX, USA) for CAB and RIL quantification. The chromatogram with well resolved peaks and mass transitions can be seen in the Figs. 1 and 2.

Mass spectrometry conditions

The optimized parameters used for the analysis are tabulated as follows:

Parameter	Value
Ionization Mode	Positive ion ESI
Collision Energy (CE)	14 V
Declustering Potential (DP)	40 V
Cell Exit Potential (CXP)	7 V
Collision Gas Pressure	Nitrogen
Ion Spray Voltage	5500 V
Source Temperature	550°C
Drying Gas Temperature	120-250°C
Curtain Gas	10 L/min
GS1 (Sheath Gas Flow)	50 L/min
GS2 (Auxiliary Gas Flow)	50 L/min
Drying Gas Flow Stream	5 L/min
Entrance Potential	10 V
Dwell Time	1 sec

Sample preparation

Protein precipitation extraction was employed for plasma sample preparation. The following procedure was followed:

1. Transfer 500 µL of standard stock solution into a 2 mL centrifuge tube
2. Add:
    - 200 µL of plasma
    - 10 µL of IS working solution at 1200 ng/mL concentration
    - 600 µL of ethanol to precipitate the proteins
    - Made up diluent
3. Mix the solution thoroughly using a vortex
4. Centrifuge at 4000 RPM for 15–20 min
5. Collect the supernatant and transfer it into an HPLC vial for injection into the chromatograph.

Preparation of stock and working solutions

The CAB stock solution was prepared by dissolving 6 mg of CAB in a buffer solution (pH 2.5, Triethylamine in water), which was then further diluted with the same buffer, resulting in a final concentration of 1.2 mg/mL.

In a similar manner, the RIL stock solution was made by dissolving 8 mg of RIL in the same buffer solution, which was then diluted serially to get a final concentration of 0.8 mg/mL.

For the IS solution, 6 mg of Raltegravir was dissolved in a 100 mL volumetric flask and diluted with the buffer solution to achieve a final concentration of 1200 ng/mL.

Working solutions

The stock solutions of CAB and RIL were serially diluted to prepare working solutions with concentrations required.

CC and quality control (QC) samples

CC of CAB (15, 45, 75, 150, 225, 300, 375, 450, and 600 ng/mL) and RIL (10, 30, 50, 100, 150, 200, 250, 300, and 400 ng/mL).

QC samples were prepared at the following concentrations:

- Low QC (LQC): 15 ng/mL for CAB; 10 ng/mL for RIL
- Medium QC (MQC): 300 ng/mL for CAB; 200 ng/mL for RIL
- High QC (HQC): 450 ng/mL for CAB; 300 ng/mL for RIL

Samples are stored at −20°C until analysis.

Method validation [30]

Validated as per US FDA guidelines, focusing on key performance metrics. To assess selectivity, blank plasma samples were scrutinized to find the separation of CAB and RIL from any endogenous components. Linearity was evaluated by constructing calibration curves from plasma samples spiked with known quantities of CAB and RIL, ensuring a regression coefficient (r^2) >0.999. Precision and accuracy were determined by analyzing QC samples at various concentrations on different days, with accuracy calculated by comparing the measured concentrations to the expected values. Recovery, matrix effects were investigated as per prescribed protocols. Stability was evaluated under several conditions, and the same are tabulated and discusses.

Pharmacokinetic study application

Studies were performed on female Sprague-Dawley rats. Analysis done at multiple time points following parenteral administration of 10 mg/kg CAB and 15 mg/kg RIL. Plasma samples were processed and analyzed to determine the concentration of CAB and RIL in plasma. Pharmacokinetic parameters, such as half-life ($t_{1/2}$), T_{max}, C_{max}, and area under the curve (AUC), and were calculated using Phoenix WinNonlin software.

Statistical analysis

All data are presented as mean ± standard deviation (SD), unless otherwise stated. Calibration curves were constructed using weighted ($1/x^2$) least-squares linear regression. For validation, accuracy (%bias)

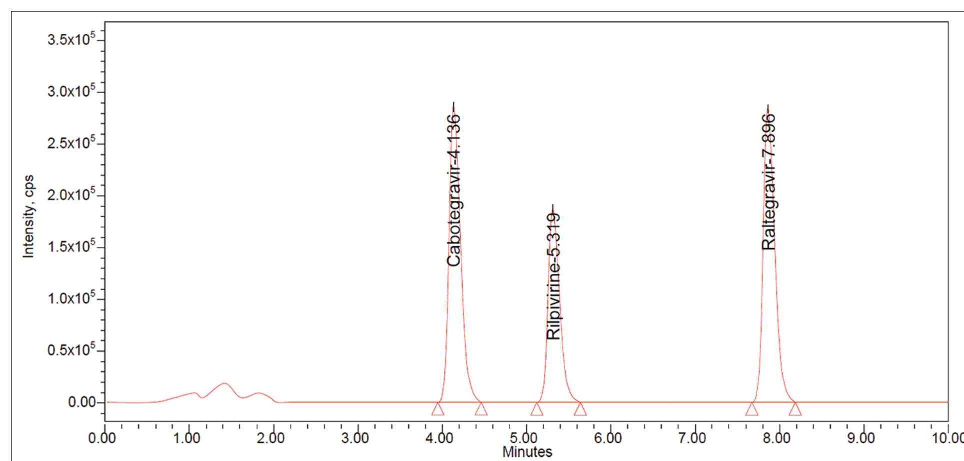

Fig. 1: Representative chromatograms of the optimized method

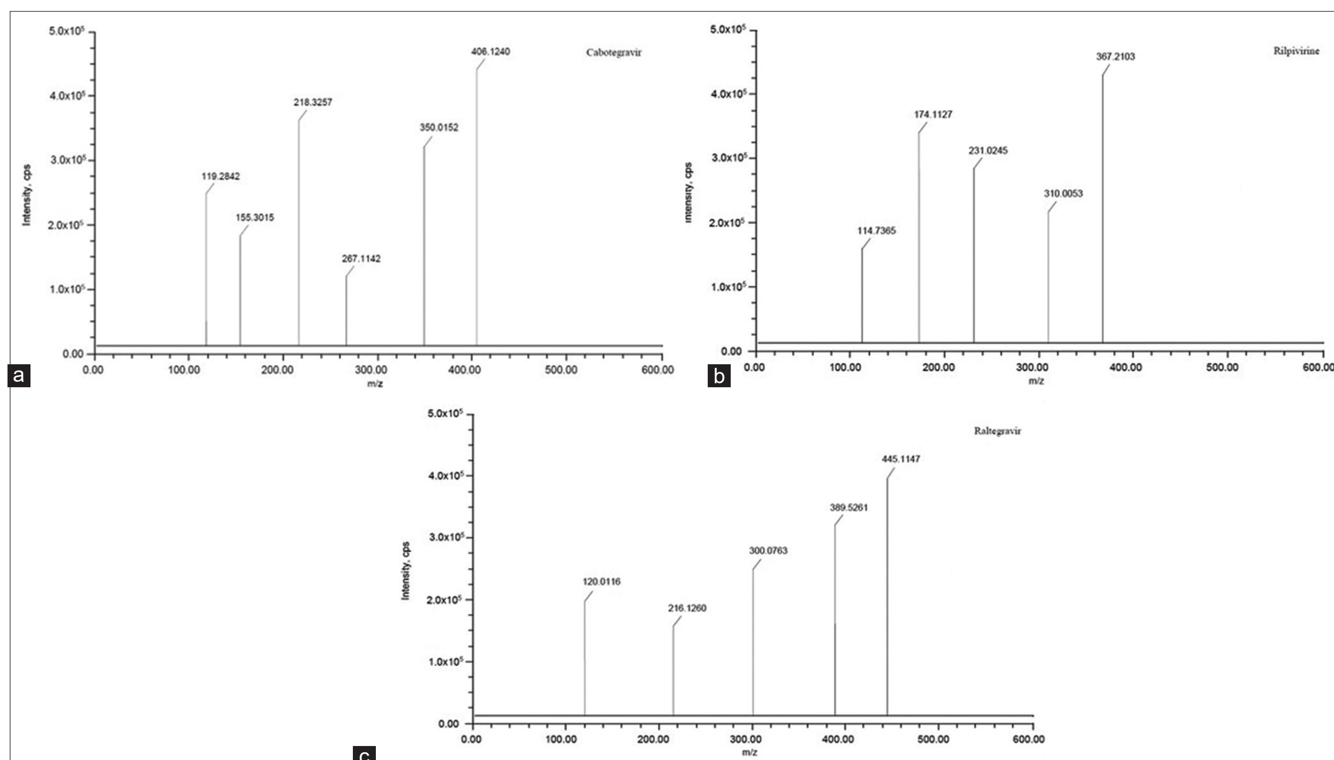

Fig. 2: Mass transitions of (a) Cabotegravir, (b) Rilpivirine, and (c) Raltegravir (internal standard)

Table 1: Calibration standards for CAB and RIL in rat plasma

Conc. CAB (ng/mL)	CAB peak areas in cps×10^5	Conc. RIL (ng/mL)	RIL peak areas in cps×10^5	Peak area ratio (CAB/IS)	Peak area ratio (RIL/IS)
15	0.142	10.00	0.094	0.05	0.033
45	0.425	30.00	0.288	0.15	0.101
75	0.709	50.00	0.472	0.251	0.167
150	1.417	100.00	0.915	0.499	0.322
225	2.126	150.00	1.417	0.749	0.499
300	2.834	200.00	1.889	1.001	1.002
375	3.543	250.00	2.361	1.244	1.335
450	4.251	300.00	2.834	1.503	1.503
600	5.668	400.00	3.778	2.004	2.004
Slope				0.0033	0.0033
Intercept				0.00013	0.00009
R^2 Value				0.99998	0.99991

Values represent mean±SD (n=6)

Table 2: Quality control samples for CAB and RIL

QC level	CAB (ng/mL)	RIL (ng/mL)	Precision (CV%)	Accuracy (%)
LQC	15	10	1.01	95.44
MQC	300	200	0.19	97.14
HQC	450	300	0.08	98.38

Values represent mean±SD (n=6), LQC: Low quality control, MQC: Medium quality control, HQC: High quality control, CAB: Cabotegravir, RIL: Rilpivirine

and precision (%CV) were evaluated at the lower limit of quantitation (LLOQ), LQC, MQC, and HQC levels for both within-run and between-run data. Acceptance criteria were ±15% (±20% for LLOQ) for accuracy and ≤15% CV (≤20% for LLOQ) for precision.

Pharmacokinetic parameters, including $AUC_0–_t$, $AUC_0–\infty$, t½, C_{max}, and T_{max}, were calculated by non-compartmental analysis using Phoenix WinNonlin (v8.3).

Comparisons between CAB and RIL were performed using two-way repeated-measures analysis of variance (ANOVA) with Drug (2 levels) and Time as within-subject factors, followed by Holm-adjusted pairwise contrasts when interactions were significant. Normality of residuals was assessed using the Shapiro–Wilk test; when assumptions were violated, a rank-based mixed-model approach was applied. T_{max} values were compared using the Wilcoxon signed-rank test, and other pharmacokinetic parameters (C_{max}, $AUC_0–_t$, $AUC_0–\infty$, t½) were compared using paired *t*-tests or Wilcoxon tests, as appropriate. A two-sided significance level of α = 0.05 was used throughout.

RESULTS

Method development

The chromatographic conditions were optimized to ensure efficient separation and minimal retention time, achieving a total run time of 10 min. The Kromasil C18 column was selected for its ability to provide optimal resolution between CAB, RIL, and the IS, Raltegravir. The mobile phase composed of 30% ethanol and 70% 0.1% triethylamine

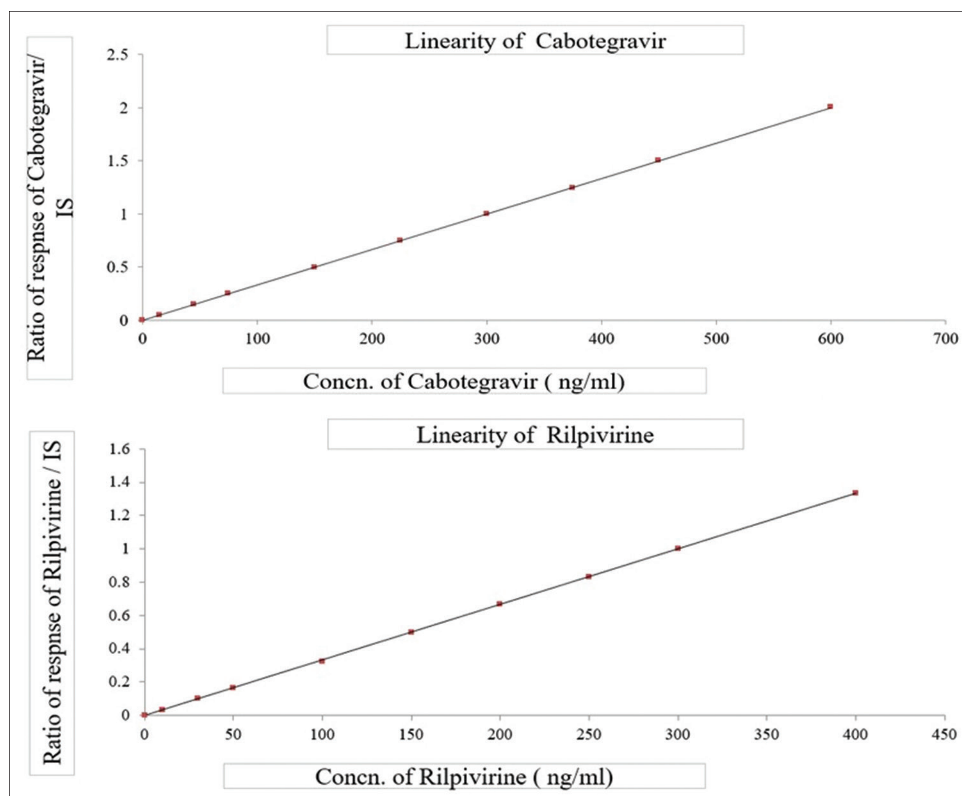

Fig. 3: Linearity plots of Cabotegravir and Rilpivirine

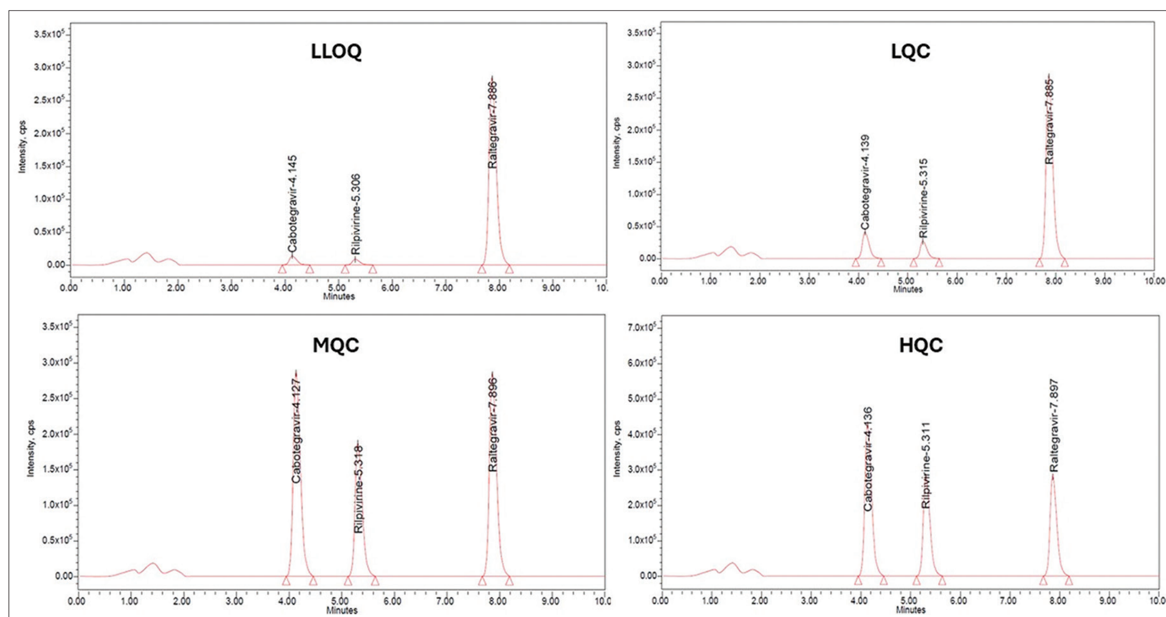

Fig. 4: Chromatograms representing different levels of concentrations

Table 3: Precision and accuracy for CAB and RIL

QC level	Drug	Within the-day precision (%)	Within the-day accuracy (%)	In between days precision (%)	In between days accuracy (%)
LQC	CAB	0.08	98.38	0.19	97.14
	RIL	0.11	97.63	0.16	98.78
MQC	CAB	0.19	97.14	0.12	97.2
	RIL	0.15	98.14	0.22	98.83
HQC	CAB	0.08	98.38	0.1	98.55
	RIL	0.13	97.28	0.21	97.19

Values represent mean±SD (n=6). LQC: Low quality control, MQC: Medium quality control, HQC: High quality control, CAB: Cabotegravir, RIL: Rilpivirine

buffer (pH 2.5) was chosen for its compatibility with both drugs and efficient elution of the analytes.

The ionization conditions for mass spectrometry were optimized to ensure high sensitivity and selectivity. The SCIEX QTRAP 5500 system in +ve electrospray ionization mode with multiple reaction monitoring provided the best sensitivity for the analytes. The transitions for CAB (m/z 406.1240 → 218.3257), RIL (m/z 367.2103 → 174.1127), and Raltegravir (IS, m/z 445.1147 → 389.5261) were optimized for maximum signal intensity and minimal background noise, ensuring a reliable detection system for trace drug levels in plasma samples.

Method validation

Selectivity and specificity

Analyzed using six different plasma lots. No significant interference was observed from endogenous substances at the retention times of CAB, RIL, and the IS (Raltegravir). The peaks for CAB, RIL, and IS were well-resolved with minimal background noise, confirming the specificity of the method. These findings demonstrate the method's suitability for analysis in complex biological matrices, ensuring that the quantification of CAB and RIL is not compromised by matrix effects.

Linearity

Calibration curves for both CAB and RIL were 15–600 ng/mL for CAB and 10–400 ng/mL for RIL. Achieved (r^2) >0.999 for both drugs, confirming that the method provides accurate quantification over a wide concentration range. The linearity of the method ensures its suitability for both low and high concentration plasma samples, which is essential for pharmacokinetic studies where drug concentrations can vary significantly (Tables 1 and 2, Fig. 3).

Precision and accuracy

Results for CAB and RIL were within acceptable limits (≤15%), with accuracy ranging from 85% to 115% at all QC levels, confirming the method's reliability for simultaneous quantification in rat plasma. One-way ANOVA was applied to evaluate intra-day and inter-day precision data across QC levels. No significant difference was observed between means ($p>0.05$), confirming reproducibility of the analytical method (Table 3 and Fig. 4).

Recovery

The parameter was assessed by standard methodology. The average recovery for CAB ranged from 94.5% to 98%, and for RIL, it ranged from 95% to 98.5%, demonstrating efficient extraction and minimal loss of analytes during sample preparation. These high recovery rates indicate the robustness of the extraction method, ensuring the required results (Table 4).

Matrix effect

After evaluating this parameter, found effects were negligible, with no significant interference observed from the plasma matrix. This

Table 4: Recovery and matrix effect for CAB and RIL

QC level	Analyte	Recovery (%)	Matrix effect (%)	Mean recovery (%)	%CV for recovery
LQC	CAB	97.01	98.85	97.01	0.08
	RIL	98.09	97.28	98.09	0.09
MQC	CAB	98.17	97.63	98.17	0.11
	RIL	97.93	97.28	97.93	0.12
HQC	CAB	95.44	96.14	95.44	0.14
	RIL	95.34	95.23	95.34	0.13

Values represent mean±SD (n=6). LQC: Low quality control, MQC: Medium quality control, HQC: High quality control, CAB: Cabotegravir, RIL: Rilpivirine

Table 5: Combined stability data for CAB and RIL

QC level	Analyte	Nominal Conc. (ng/mL)	Mean peak area (×10^5)	SD	%CV	Mean accuracy (%)
Bench-top stability of Cabotegravir (CAB) and Rilpivirine (RIL)						
LQC	CAB	45	0.411	0.00366	0.89	96.61
	RIL	30	0.271	0.00264	0.97	95.69
MQC	CAB	300	2.795	0.00387	0.14	98.55
	RIL	200	1.837	0.00394	0.21	97.3
HQC	CAB	450	4.135	0.00427	0.1	97.2
	RIL	300	2.796	0.00408	0.15	98.73
Freeze–Thaw stability of Cabotegravir (CAB) and Rilpivirine (RIL)						
LQC	CAB	45	0.412	0.00392	0.95	96.85
	RIL	30	0.27	0.00214	0.79	95.34
MQC	CAB	300	2.802	0.0028	0.1	98.8
	RIL	200	1.835	0.00437	0.23	97.19
HQC	CAB	450	4.127	0.00405	0.1	97.01
	RIL	300	2.779	0.00539	0.19	98.13
Autosampler stability of Cabotegravir (CAB) and Rilpivirine (RIL)						
LQC	CAB	45	0.405	0.00493	1.22	95.2
	RIL	30	0.274	0.00447	1.63	96.75
MQC	CAB	300	2.755	0.0035	0.13	98.17
	RIL	200	1.838	0.00437	0.16	97.62
HQC	CAB	450	4.185	0.00564	0.13	98.38
	RIL	300	2.757	0.00607	0.22	97.35
Long-term stability of Cabotegravir (CAB) and Rilpivirine (RIL) at HQC level						
Day-1	CAB	450	4.185	0.00341	0.08	98.38
	RIL	300	2.749	0.00314	0.11	97.07
Day-7	CAB	450	4.006	0.00409	0.1	94.17
	RIL	300	2.697	0.00306	0.11	95.23
Day-14	CAB	450	3.905	0.00314	0.08	91.8
	RIL	300	2.505	0.00306	0.12	90.22
Day-21	CAB	450	3.725	0.00341	0.09	94.17
	RIL	300	2.505	0.00314	0.18	91.37
Day-28	CAB	450	3.625	0.00454	0.13	85.21
	RIL	300	2.403	0.00306	0.12	88.45

Values represent mean±SD (n=6). Peak areas are expressed in counts per second (cps×10^5). CAB: Cabotegravir, RIL: Rilpivirine, LQC: Low quality control, MQC: Medium quality control, HQC: High quality control

finding further supports the reliability and specificity of the method, confirming that the ionization of CAB and RIL is not affected by the biological matrix.

Table 6: Sensitivity results (LLOQ) for CAB and RIL

Replicate number	Nominal concentration of CAB (ng/mL)	Nominal concentration of RIL (ng/mL)	CAB peak area	RIL peak area
1	15	10	0.135×10^5	0.088×10^5
2	15	10	0.131×10^5	0.089×10^5
3	15	10	0.136×10^5	0.086×10^5
4	15	10	0.137×10^5	0.088×10^5
5	15	10	0.133×10^5	0.085×10^5
6	15	10	0.132×10^5	0.086×10^5
Mean	15	10	0.134×10^5	0.087×10^5
SD			0.00237	0.00155
%CV			1.77	1.78
Accuracy			94.50%	92.16%

Values represent mean±SD (n=6). CAB: Cabotegravir, RIL: Rilpivirine, LLOQ: Lower limit of quantitation

Table 7a: Plasma drug concentrations of CAB and RIL

Time intervals (h)	Cabotegravir average Conc. (ng/mL)	Rilpivirine average Conc. (ng/mL)
0.5	65.103±1.076	36.935±0.833
1.0	134.791±1.112	82.097±1.047
2.0	247.664±1.096	160.434±1.040
3.0	294.181±2.056	193.379±1.708
5.0	134.527±2.216	79.537±1.370
7.0	57.750±0.982	29.749±1.015
9.0	21.478±1.461	12.694±1.054
12.0	0.000	0.000

CAB: Cabotegravir, RIL: Rilpivirine

Table 7b: Pharmacokinetic parameters for CAB and RIL

Parameter	CAB (mean±SD)	RIL (mean±SD)	p-value
AUC_{0-t} (ng.h/mL)	1261±1.21 ng.h/mL	781±0.21 ng.h/mL	<0.01
C_{max} (ng/mL)	294.2±0.87 ng/mL	193.4±1.12 ng/mL	<0.01
$AUC_{0-\infty}$ (ng.h/mL)	1261±1.12 ng.hr/mL	781±0.87 ng.h/mL	<0.01
T_{max} (h)	3.0 h	3.0 h	>0.05
$T_{1/2}$ (h)	9.0 h	9.0 h	>0.05

Values represent mean±SD (n=6)

Stability

The analytes were stable under bench-top conditions (5 h), freeze-thaw cycles (3 cycles), and long-term storage (−20°C for 23 days). The stability of the drugs at low concentrations (LLOQ) was also confirmed, which is crucial for pharmacokinetic studies involving multiple sample collections over time (Table 5).

Sensitivity

The method demonstrated excellent sensitivity, making the method highly suitable for pharmacokinetic studies, where drug concentrations in plasma may be low, particularly during the elimination phase or at early time points following drug administration. The sensitivity of the method ensures that even trace levels of CAB and RIL can be accurately quantified, providing reliable data for pharmacokinetic modeling (Table 6).

Pharmacokinetic application

Applied to pharmacokinetic studies of CAB and RIL in Sprague-Dawley rats. Plasma samples were collected at multiple time points following the parenteral administration of 10 mg/kg CAB and 15 mg/kg RIL. The concentration of CAB and RIL in plasma was determined using the LC-MS/MS method, and pharmacokinetic parameters, such as C_{max}, T_{max}, AUC, and half-life ($t_{1/2}$) were calculated. The results of these studies will provide critical insights into the pharmacokinetic behavior of CAB and RIL *in vivo* and support future clinical studies.

The plasma concentration–time profiles of CAB and RIL revealed comparable absorption and elimination characteristics, with both agents achieving peak plasma concentrations (T_{max}) at 3 h. CAB demonstrated a significantly higher maximum plasma concentration (C_{max}: 294.2 ± 0.87 ng/mL) relative to RIL (193.4 ± 1.12 ng/mL). The systemic exposure, as indicated by AUC_{0-t} and $AUC_{0-\infty}$, was also greater for CAB (1261 ng·h/mL) compared to RIL (781 ng·h/mL). CAB showed a significantly higher C_{max} and AUC_0-_t values than RIL (p<0.01), whereas T_{max} and $t_{½}$ did not differ significantly (p>0.05), indicating similar elimination kinetics between the two drugs. Despite the observed differences in exposure and C_{max}, both drugs exhibited an identical elimination half-life ($T_{1/2}$) of 9.0 h, suggesting similar clearance kinetics. Collectively, these findings indicate that CAB achieves superior plasma exposure while maintaining pharmacokinetic parameters consistent with RIL in terms of absorption time and elimination rate (Table 7a and b, Fig. 5).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (v9.0). Data are expressed as mean ± standard deviation (SD). Normality of data was verified by the Shapiro–Wilk test. Inter-group differences between CAB and RIL plasma concentrations at each time point were analyzed

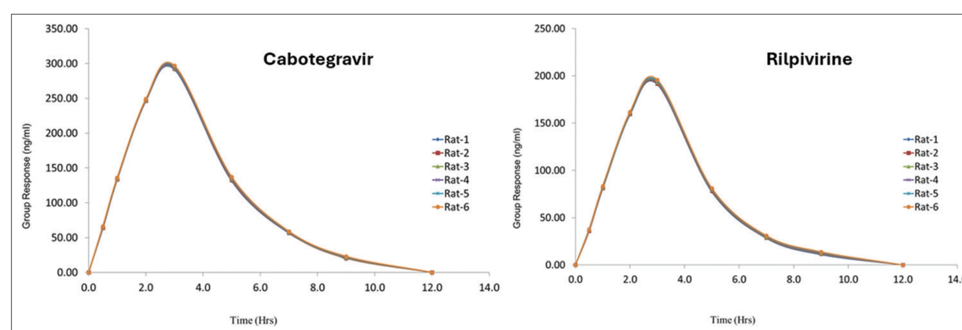

Fig. 5: Plasma concentration–time profiles of Cabotegravir (CAB) and Rilpivirine (RIL) in rats. The mean plasma drug concentrations of CAB (left) and RIL (right) were monitored over a 12-h period following administration. Both drugs exhibited rapid absorption with peak plasma levels (T_{max}) attained at approximately 3 h, followed by a gradual decline in concentration. CAB achieved a higher maximum plasma concentration (C_{max} ≈ 294 ng/mL) compared to RIL (C_{max} ≈ 193 ng/mL), while both showed similar elimination kinetics with measurable drug levels detectable up to 12 h. Data represent the response from six rats (n=6), with individual animal trends shown. Statistical comparison confirmed significantly higher Cmax and AUC_0-_t for CAB (p<0.01), while T_{max} and $t_{½}$ were comparable (p>0.05)

using unpaired Student's *t*-test. Pharmacokinetic parameters (C_{max}, AUC_{0-t}, t½, and T_{max}) were compared using one-way ANOVA followed by Tukey's *post hoc* test, with p<0.05 considered statistically significant.

Comparison with existing methods

To date, several analytical methods have been reported; however, these methods either focus on a single drug or lack the necessary validation for accurate and simultaneous quantification in rat plasma. The method developed in this study is unique in its ability to quantify both CAB and RIL concurrently, with high precision and sensitivity, and is validated, ensuring compliance with industry standards [30,31].

DISCUSSION

The results demonstrate that the LC-MS/MS method provides a reliable, accurate, and appropriate for the quantification of CAB and RIL in rat plasma. The optimization of chromatographic and mass spectrometry conditions has resulted in efficient separation, high sensitivity, and excellent resolution of both drugs and the IS. The method's validation, in accordance with FDA guidelines, ensures its robustness, precision, and accuracy. In addition, the method's successful application in pharmacokinetic studies provides further evidence of its utility in assessing the pharmacokinetic profiles of CAB and RIL. Statistical analysis confirmed that CAB attained significantly higher systemic exposure compared with RIL (p<0.01), though both drugs exhibited similar elimination rates.

By offering a reliable analytical method for the simultaneous quantification of both drugs, this study provides detailed insights into the pharmacokinetics of CAB and RIL in HIV therapy. The method is not only suitable for research applications but also holds potential for clinical studies, drug development, and combination therapy monitoring in HIV patients.

CONCLUSION

The method demonstrated excellent chromatographic resolution, high sensitivity, and selectivity, making it highly suitable for pharmacokinetic studies. Validation according to US FDA guidelines. The method also exhibited minimal matrix effects and high extraction efficiency, ensuring reliable quantification of CAB and RIL at low concentrations.

The successful application of this method in pharmacokinetic studies underlines its potential for assessing the *in vivo* behavior of both drugs, which is crucial for understanding their pharmacokinetic profiles, especially in the context of combination therapy. This method not only supports future pre-clinical and clinical research on CAB and RIL but also offers a valuable tool for evaluating the pharmacokinetics of other drugs in similar therapeutic areas. The method's high sensitivity and reproducibility make it a robust analytical approach for drug development and therapeutic monitoring, particularly in HIV treatment regimens.

Overall, this LC-MS/MS method is a valuable addition to the field of bioanalysis, providing a reliable and efficient tool for the simultaneous estimation of CAB and RIL in biological matrices, and facilitating further research into their combined use in HIV treatment.

AUTHOR CONTRIBUTIONS

Ramya Nagabathula has contributed to method development, validation, data collection, analysis, and manuscript writing, while Kamepalli Sujana has contributed to study design, provided guidance on data analysis, and revised the manuscript.

DECLARATION OF COMPETING INTEREST

All the authors declare that they do not have any conflicts of interest.

AUTHOR FUNDING

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