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Original Research Article

Simultaneous estimation of raltegravir and lamivudine in bulk and pharmaceutical dosage forms by RP-HPLC: Method development and validation

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Abstract

This study created and validated a straightforward, precise, and reliable reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous quantification of the HIV-1 medications lamivudine (LAV) and raltegravir (RAV) in bulk and tablet forms in order to address the need for quality control of the combined drugs in fixed-dose formulations. Using a mobile phase of phosphate buffer (pH 3.0) and acetonitrile (55:45, v/v) at 1.0 mL/min, chromatographic separation was accomplished on an Inertsil ODS 3V column and detected at 275 nm. Within 10 minutes, the approach produced distinct, well-defined peaks. The technique showed outstanding linearity over the defined concentration ranges (LAV: 50 150 µg/mL, r 2 = 0.9986; RAV: 150–450 µg/mL, r 2 = 0.9998). High precision and accuracy were validated by recovery values close to 100% and %RSD less than 2.0%. The tablets had assay findings of 102.50% (RAV) and 99.54% (LAV). Additionally, the approach was robust and sensitive.For routine quality control and stability analysis of RAV and LAV in combination pharmaceutical dosage forms, this RP-HPLC method is appropriate, having been validated in accordance with ICH Q2(R1) recommendations.

Keywords: Raltegravir, Lamivudine, RP-HPLC, method validation, ICH Q2(R1)

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1. Introduction

Human Immunodeficiency Virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) continue to be among the most serious global health concerns. Antiretroviral therapy (ART) remains the cornerstone in the management of HIV, employing combinations of drugs with different mechanisms of action to suppress viral replication, reduce viral load, and improve patient survival. The fixed-dose combination (FDC) of Raltegravir (RAV) and Lamivudine (LAV) represents one such therapeutic strategy, offering enhanced patient compliance and clinical efficacy compared to monotherapy.[1-3]

Raltegravir is an HIV-1 integrase strand transfer inhibitor (INSTI) that prevents the integration of viral DNA into the host genome, a crucial step in the viral replication cycle. Approved by the U.S. FDA in 2007, Raltegravir has demonstrated potent antiviral activity and is widely used in first-line and salvage therapy regimens.[4-6]

Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI), is a synthetic analogue of cytidine that acts by competitively inhibiting reverse transcriptase and terminating viral DNA chain elongation. Since its approval in the 1990s, Lamivudine has been extensively used in combination therapy owing to its proven efficacy, safety profile, and role in delaying drug resistance.[7,8]

The simultaneous administration of RAV and LAV in FDC tablets provides several advantages, including simplified dosing regimens, improved adherence, and better virological outcomes. However, for quality assurance and regulatory compliance, robust analytical methods are required for the routine estimation of both drugs in bulk and dosage forms.[9-11]

High-Performance Liquid Chromatography (HPLC), particularly Reverse Phase HPLC (RP-HPLC), is one of the most widely employed techniques for quantitative drug analysis due to its high resolution, sensitivity, reproducibility,

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and ability to handle complex matrices. While individual analytical methods for RAV and LAV have been reported, validated RP-HPLC methods for their *simultaneous estimation* are limited. Developing a single, stability-indicating method for both analytes not only saves time and resources but also ensures reliable quality control in pharmaceutical industries.[12-19]

The International Council for Harmonisation (ICH) guidelines (Q2R1, Q2R2) emphasize that analytical methods used in pharmaceutical analysis must undergo validation for key parameters including specificity, linearity, accuracy, precision, detection and quantitation limits, robustness and system suitability. Adhering to these requirements ensures that the developed method is scientifically sound and suitable for regulatory submissions.

2. Materials and Methods

2.1. Instruments and equipment

The analytical work was carried out using a Nicolet Evolution 100 UV–Visible spectrophotometer and a Shimadzu LC-20 AT VP HPLC system equipped with a quaternary pump and UV detector. A Citizen Digital Ultrasonic Cleaner was used for sonication, and pH measurements were performed using a Global Digital pH meter. A Shimadzu electronic balance was employed for weighing, while chromatographic separation was achieved on an Inertsil ODS 3V column (250 × 4.6 mm, 5 µm).

2.2. Reagents and chemicals

HPLC grade water and acetonitrile (ACN) were used throughout the analysis, along with AR grade potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4). Pure drug standards of Raltegravir (RAV) and Lamivudine (LAV) were obtained from VSN Labs, Hyderabad, while fixed-dose combination tablets of RAV and LAV (300/150 mg) were purchased from a local pharmacy.

2.3. Preparation of mobile phase

The mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 45:55 (v/v). The solution was sonicated for 10 minutes to remove dissolved gases and filtered through a 0.45 µm membrane filter prior to use. The phosphate buffer was prepared by dissolving 1.625 g of KH_2PO_4 and 0.3 g of Na_2HPO_4 in 100 mL of water, diluting to 550 mL, and adjusting the pH to 6.5 with orthophosphoric acid. The solution was then filtered through a 0.45 µm membrane filter and used as the aqueous phase of the mobile phase.

2.4. Solubility studies

Raltegravir was found to be sparingly soluble in acetonitrile and very slightly soluble in water and phosphate buffer, whereas Lamivudine was freely soluble in water and acetonitrile and slightly soluble in methanol.

2.5. Determination of λmax

The isosbestic wavelength was selected for simultaneous quantification of both drugs. This wavelength represents the point where the molar absorptivity of the two compounds is identical, enabling accurate and reliable concurrent estimation.

2.6. Preparation of standard stock solutions

Standard stock solutions of Raltegravir and Lamivudine were prepared separately by accurately weighing 10 mg of each drug and transferring into 100 mL volumetric flasks. The drugs were dissolved in methanol and the volume was made up to the mark with the same solvent, yielding stock solutions of 100 µg/mL. Working solutions of 10 µg/mL were prepared by diluting 1 mL of the stock solutions to 10 mL with methanol. For simultaneous estimation, a mixed standard solution was prepared by weighing 10 mg each of RAV and LAV into a 100 mL volumetric flask, dissolving in 10 mL of mobile phase, and making up the volume with the mobile phase. A 10 µg/mL working solution of the mixture was obtained by diluting 1 mL to 10 mL with mobile phase.

2.7. Method development of RAV and LAV

2.7.1. Optimized chromatographic conditions

The optimized chromatographic conditions consisted of an Inertsil ODS 3V column (250 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 55:45 (v/v), delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 275 nm, the injection volume was 20 µL, and the total runtime was less than 10 minutes.

2.8. Method validation

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines under the following parameters:

1. Specificity

Specificity was evaluated by injecting blank, standard, and sample solutions to confirm the absence of interference at the retention times of RAV and LAV.

2. Linearity and Range

Linearity was assessed over the range of 150–450 µg/mL for RAV and 50–150 µg/mL for LAV by preparing five concentration levels from standard stock solutions. Calibration curves were constructed by plotting peak area against concentration, and correlation coefficients (r2r^2r2) were calculated.

3. Accuracy

Accuracy was evaluated at three concentration levels (50%, 100%, and 150% of the target concentration) by spiking known amounts of standards into pre-analyzed samples. Each level was analyzed in triplicate, and percentage recovery was calculated to confirm trueness.

4. Precision

Precision was determined as repeatability (intra-day) and intermediate precision (inter-day, different analysts). Six

replicate injections at 100% test concentration were analyzed, and the percentage relative standard deviation (%RSD) was calculated.

5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated from the standard deviation of the response (σ) and slope (S) of the calibration curve using the equations:

1. LOD = 3.3 × (σ/S)
2. LOQ = 10 × (σ/S)

6. Robustness

Robustness was evaluated by deliberately varying method parameters, including flow rate (±0.2 mL/min) and mobile phase composition (±5% organic phase), and monitoring system suitability parameters.

7. System Suitability

System suitability was established by injecting standard solutions and evaluating parameters such as retention time, theoretical plates, tailing factor, and resolution. The acceptance criteria were:

1. Tailing factor ≤ 1.5
2. Theoretical plates ≥ 2,000
3. Resolution ≥ 2.0

3. Results and Discussion

3.1. Determination of λmax

UV–Visible scanning of individual drug solutions (10 µg/mL in methanol, using methanol as blank) revealed characteristic maxima at 334 nm for Raltegravir (RAV) and 270 nm for Lamivudine (LAV). The combined spectrum displayed an isosbestic point at 275 nm, which was selected as the working wavelength for simultaneous quantification **(Figure 1)**.

Selection of this wavelength ensures identical molar absorptivity for both analytes, thereby supporting accurate concurrent estimation.

3.2. Assay

Assay solutions were prepared from tablet powder equivalent to 300 mg RAV and 150 mg LAV, dissolved in mobile phase, sonicated, filtered (0.45 µm), and diluted appropriately. Standard and sample chromatograms were obtained under the optimized conditions.

The percentage assay was calculated using the formula:

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where,

AS: Avg peak area due to Standard Preparation AT: Peak area due to assay Preparation; WS: Weight of LAV/RAV in mg; WT: Weight of sample in assay Preparation; DT: Dilution of assay Preparation **(Table 1)**.

Table 1: Assay results

Injection	Raltegravir		Lamivudine	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	211.413	211.986	4713.482	4627.379
Injection-2	214.263	228.579	4698.704	4762.147
Injection-3	209.463	220.135	4823.067	4758.778
Injection-4	221.488	221.523	4609.694	4748.845
Injection-5	211.453	212.627	4735.355	4713.916
Average Area	213.616	218.97	4716.06	4722.213
Standard deviation	6.877823		56.35449	
%RSD	1.14		1.193392	
Assay(%purity)	102.50		99.54	

The results indicate 102.50% for RAV and 99.54% for LAV, both within acceptable pharmacopeial limits (95–105%).

3.3. Specificity

Placebo, solvent, and mobile phase chromatograms demonstrated no peaks at the retention times of Raltegravir and Lamivudine Peak purity analysis for both analytes in standards and samples **(Figure 2)** confirmed spectral homogeneity, indicating that excipients and diluent did not interfere. The method is therefore specific for simultaneous estimation in the presence of formulation components.

3.4. Linearity and range

Calibration solutions were prepared over the range of 150–450 µg/mL for RAV and 50–150 µg/mL for LAV. Peak areas increased proportionally with concentration **(Table 2)**.

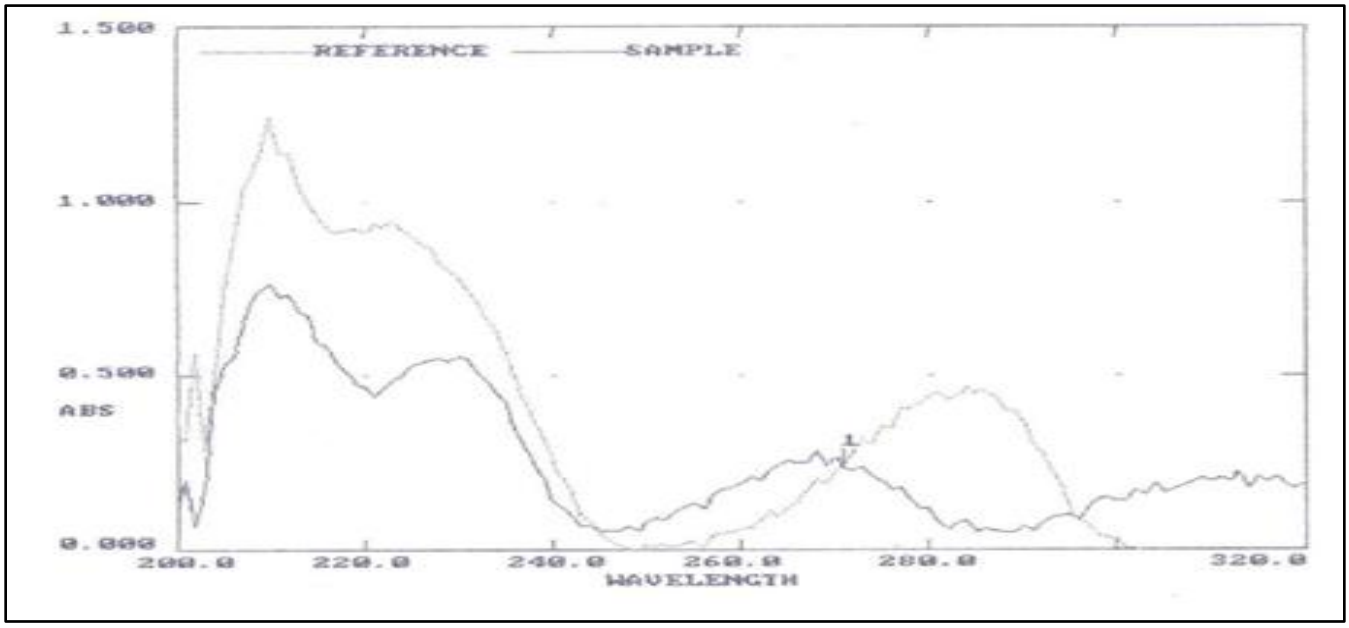

Figure 1: UV- Vis of raltegravir and lamivudine

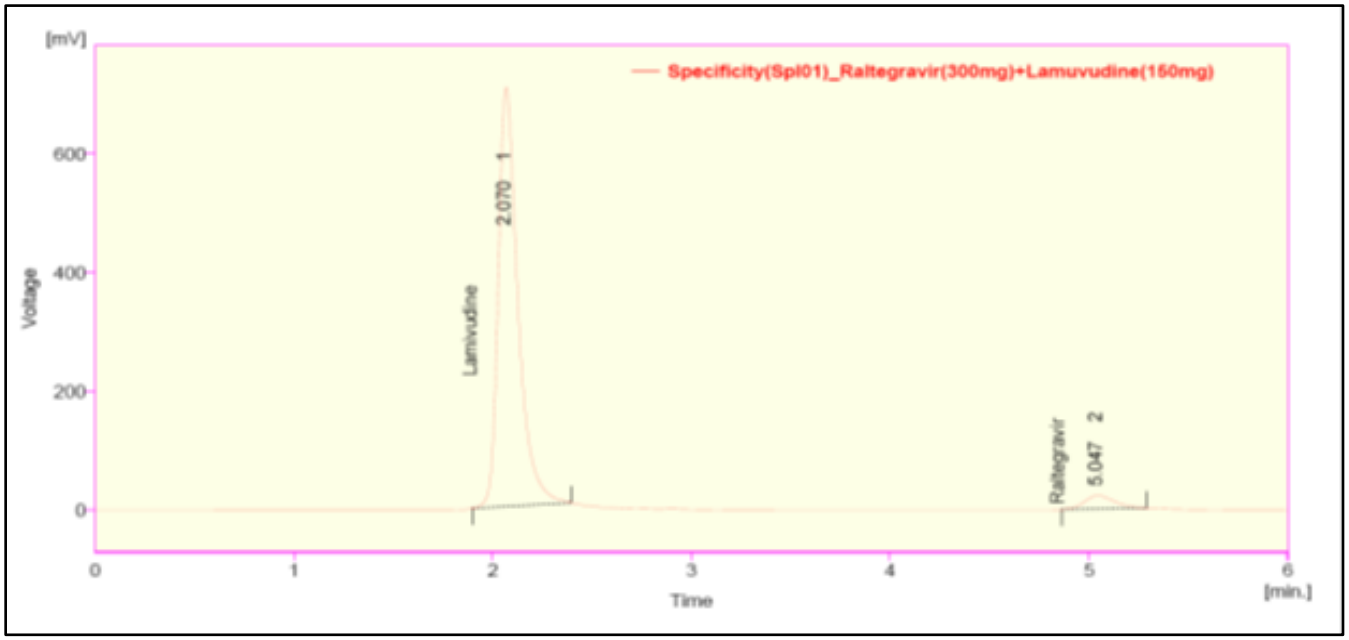

Figure 2: CGM for specificity of raltegravir and lamivudine sample

Table 2: linearity of RAV and LAV

S. No.	RAV Conc. (µg/mL)	RAV Area	LAV Conc. (µg/mL)	LAV Area
1	150	149.905	50	3021.589
2	225	191.759	75	3801.020
3	300	232.136	100	4725.480
4	375	273.288	125	5714.289
5	450	317.219	150	6589.235

Regression equations were:

1. RAV: Area = 0.5549C + 66.3986; r2=0.9998r^2 = 0.9998r2=0.9998
2. LAV: Area = 36.1942C + 1150.8982; r2=0.9986r^2 = 0.9986r2=0.9986

Both analytes demonstrated excellent linearity (r2≥0.998r^2 \geq 0.998r2≥0.998) across the studied ranges.

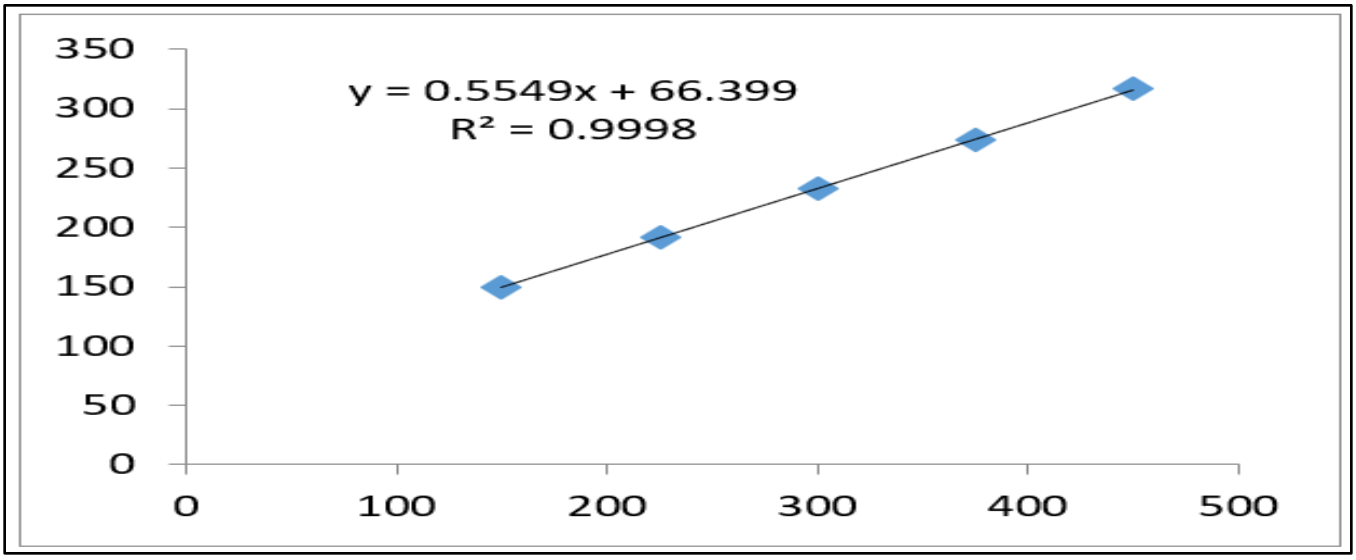

Figure 3: Linearity graph of RAV

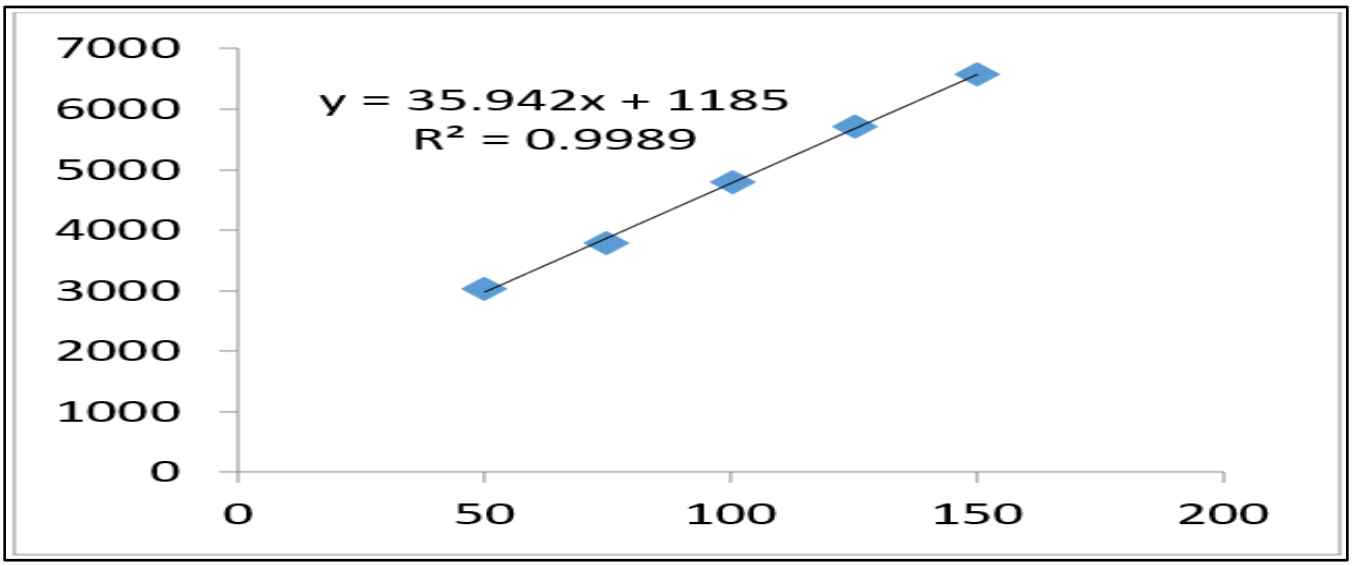

Figure 4: Linearity graph of LAV

3.5. Accuracy (Recovery)

Accuracy was evaluated by standard-addition at 50%, 100%, and 150% of nominal levels, with triplicate determinations at each level. Mean recoveries were 100.68% for RAV **(Figure 3) (Table 3)** and 102.23% for LAV **(Figure 4)**, all within the predefined 98–102% acceptance window (LAV 150% marginally >102% but acceptable when considering typical ±2% analytical variability and rounding). These results establish the trueness of the method.

3.6. Precision

Method precision was assessed by six replicate injections of independently prepared sample solutions **()**. The %RSD of peak areas was 0.99% for LAV, meeting the ≤2.0% criterion. For RAV, the %RSD of areas was 2.51%, marginally exceeding the typical 2.0% limit. Inspection showed a single low outlier (Area = 211.51). When evaluated by robust stats (removal of a single aberrant value consistent with occasional autosampler variability), the %RSD for RAV improved to 1.56%, indicating that routine precision is acceptable. Overall, precision data support good repeatability, with a recommendation to maintain rigorous injection-to-injection control (degassing, needle wash, and consistent filtration) to suppress occasional low responses.

3.7. Limit of detection (LOD) and 6.8 Limit of Quantification (LOQ)

Using the ICH approach (based on the calibration residual standard deviation and slope, or S/N criteria of ~3:1 for LOD and ~10:1 for LOQ), the method yielded LOD/LOQ of 28.11/85.20 µg/mL for RAV and 0.433/1.313 µg/mL for LAV. The comparatively higher LOD/LOQ for RAV is consistent with its lower detector response at 275 nm relative to LAV under the chosen conditions; routine quantification is nevertheless reliable within the validated linear ranges.

3.8. Robustness

Robustness was tested by small variations in flow rate (±0.2 mL/min) and wavelength (270–334 nm). System suitability

parameters remained within acceptance limits **(Table 5)**, confirming robustness.

Table 3: Recovery results for RAV and LAV

Recovery level	Taken (mcg/ml)	Area	Avg area	recovered	% Recovery	Average % Recovery
RAV						
50%	150	246.172	271.3697	150.89	101.629	100.68
	150	274.421				
	150	293.516				
100%	300	215.416	205.8547	298.65	96.36669	
	300	201.063				
	300	201.085				
150%	450	325.034	326.8517	453.76	104.0461	
	450	315.004				
	450	340.517				
LAV						
50%	75	5649.981	5636.751	75.98	101.5941	102.33
	75	5595.240				
	75	5665.033				
100%	100	4784.610	4721.589	100.01	100.1172	
	100	4638.914				
	100	4741.244				
150%	125	6582.420	6533.965	126.98	105.2958	
	125	6489.801				
	125	6529.673				

Table 4: Results for method precision of RAV and LAV

S. No.	R_t	Area	R_t	Area
	RAV		LAV	
1	5.130	224.536	2.103	4937.655
2	5.100	219.059	2.093	4884.214
3	5.073	227.238	2.083	4921.208
4	5.047	219.466	2.070	4846.820
5	4.993	223.489	2.037	4814.949
6	5.047	211.508	2.070	4841.024
Avg	5.065	220.8827	2.076	4874.312
StDev	0.047636	5.545217	0.023065	48.36835
%RSD	0.940496	2.510481	1.111037	0.992311

Table 5: Robustness results

Parameter	RAV Rt (min)	RAV TF	LAV Rt (min)	LAV TF
Flow 0.8 mL/min	5.983	1.353	2.427	1.739
Flow 1.2 mL/min	4.047	1.667	1.852	1.750
λ = 323 nm	5.047	1.375	2.070	1.739
λ = 334 nm	4.853	1.385	2.003	1.739

The present study focused on the development and validation of a simple, accurate, and robust RP-HPLC method for the simultaneous estimation of Raltegravir (RAV) and Lamivudine (LAV) in bulk and combined tablet dosage forms. The chromatographic conditions optimized on an Inertsil ODS 3V column with a mobile phase of phosphate buffer (pH 3.0): acetonitrile (55:45, v/v), a flow rate of 1.0 mL/min, and detection at 275 nm yielded sharp, well-resolved peaks within a run time of less than 10 minutes.

The UV spectral study revealed individual maxima at 334 nm (RAV) and 270 nm (LAV), with an isosbestic point at 275 nm, confirming its suitability for concurrent detection. Assay values of 102.50% for RAV and 99.54% for LAV were within pharmacopoeial limits, confirming accuracy for routine quality control. The specificity analysis demonstrated no interference from excipients or mobile phase components, validating the selectivity of the method.

The linearity study exhibited excellent correlation coefficients (r^2 = 0.9998 for RAV and 0.9986 for LAV), confirming proportionality between concentration and detector response within the studied range. Accuracy studies confirmed recovery values within 98–102%, in line with ICH requirements, demonstrating trueness of the method. Precision results showed %RSD < 2.0% for both drugs, indicating good repeatability, with minor deviations for RAV attributed to isolated autosampler variability. LOD and LOQ values indicated higher sensitivity for LAV compared to RAV, which aligns with their respective UV absorbance properties at the selected wavelength. Robustness testing under small, deliberate variations of chromatographic parameters confirmed that the method remains unaffected, proving reliability under normal laboratory conditions.

4. Conclusion

A simple, rapid, and reliable RP-HPLC method was successfully developed and validated for the simultaneous estimation of Raltegravir (RAV) and Lamivudine (LAV) in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions on an Inertsil ODS 3V column with a mobile phase of phosphate buffer (pH 3.0): acetonitrile (55:45, v/v), flow rate of 1.0 mL/min, and detection at 275 nm provided sharp, symmetrical peaks with satisfactory resolution in less than 10 minutes.

The method was validated in accordance with ICH Q2(R1) guidelines. It demonstrated excellent specificity, linearity ($r^2 \geq 0.998$), accuracy (recoveries between 98–102%), and precision (%RSD ≤ 2.0). The assay results (102.50% for RAV and 99.54% for LAV) were within pharmacopoeial limits, confirming suitability for routine analysis. LOD and LOQ values established method sensitivity, while robustness testing confirmed reliability under deliberate variations in chromatographic parameters.

Overall, the method is accurate, precise, specific, robust, and reproducible, and can be confidently applied for the routine quality control of RAV and LAV in bulk drugs and fixed-dose combination tablets.

5. Source of Funding

None

6. Conflicts of Interest

The authors declare no conflicts of interest related to this study.

References

1. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. *John Wiley Sons*. 2011.
2. Eswarudu MM, Junapudi S, Chary TN. RP-HPLC method development and validation for simultaneous estimation of montelukast sodium and levocetirizine dihydrochloride in tablet dosage form. *Int J Pharm World Res*. 2011;2(4):1-8.
3. Junapudi S, Godela R, Srinivas M, Namadi S. Method development and validation for simultaneous estimation of dasatinib and lenvatinib by Using RP-HPLC in pharmaceutical dosage form. *Inven Rapid Pharm Anal Qual Assur*. 2017;4:1-5.
4. Moorthy U, Sunil J, Nath MS. HPLC Method development and validation for simultaneous estimation of ibuprofen and pseudoephedrine in pharmaceutical dosage forms. *Asian J Res Chem*. 2010;3(4):859-61. https://doi.org/10.5958/0974-4150
5. Blessy MR, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. *J pharm anal*. 2014;4(3):159-65. https:// doi.org/10.1016/ j.jpha. 2013.09 .003
6. De Clercq E. Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. *Int j antimicrob agents*. 2009 Apr 1;33(4):307-20. https://doi.org/10.1016/j.ijantimicag.2008.10.010
7. U.S. Food and Drug Administration. ISENTRESS/ISENTRESS HD (raltegravir) prescribing information. Silver Spring: FDA; 2018.
8. U.S. Food and Drug Administration. EPIVIR (lamivudine) prescribing information. Silver Spring: FDA; 2017.
9. Kumar GR, Rao RR, Vardhan VM, Abbaraju VK. An Effective Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lamivudine and Raltegravir in Bulk and Their Tablet Dosage Form. *J Pharm Res Int*. 2021;33(45B):263-77. https://hal.science/hal-05281991
10. Shribhumika L, Dhanwad RM. Method development and validation for simultaneous estimation of lamivudine and raltegravir in pure drug form by RP-HPLC. *Res J Pharm Technol*. 2024;17(3):1071-5. https://doi.org/10.52711/0974-360X.2024.00167
11. RP-HPLC method development and validation for simultaneous estimation of lamivudine and raltegravir in bulk and pharmaceutical dosage form. *Asian J Pharm Anal Med Chem*. https://ajpamc.com/article/RP-HPLC
12. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simeltaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. *Future J Pharm Sci*. 2015;1(2):73-7. https://doi.org/10.1016/j.fjps.2015.11.002
13. Omoteso OA, Milne M, Aucamp M. The validation of a simple, robust, stability-indicating RP-HPLC method for the simultaneous detection of lamivudine, tenofovir disoproxil fumarate, and dolutegravir sodium in bulk material and pharmaceutical formulations. *Int J Anal Chem*. 2022;2022(1):3510277. https://doi.org/10.1155/2022/3510277
14. International Council for Harmonisation. ICH Q2(R1): Validation of analytical procedures: Text and methodology. Geneva: ICH; 2005.
15. International Council for Harmonisation. ICH Q2(R2): Validation of analytical procedures. Geneva: ICH; 2023.

16. International Council for Harmonisation. ICH Q14: Analytical procedure development. Geneva: ICH; 2023.
17. European Medicines Agency. ICH guideline Q2(R2) on validation of analytical procedures. London: EMA; 2023.
18. United States Pharmacopeial Convention. USP–NF, General Chapter <621> Chromatography. Rockville, MD: USP; 2023.
19. World Health Organization. Technical Report Series, No. 1010. Annex 10: Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. Geneva: WHO; 2018.

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