International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

A comprehensive review on analytical methods of Raltegravir

Pagadipally Santhosh Kumar[1], Mangam Mamatha[1], Nagireddy Roshitha[1], Anumolu DurgaPani Kumar[1], Naraparaju Swathi[2]*

[1]*Department of pharmaceutical Analysis, Gokaraju Rangaraju College of pharmacy, Hyderabad-500090, Telangana, India .*

[2]*Department of pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Hyderabad-500090, Telangana, India.*

Date of Submission: 05-07-2025 Date of Acceptance: 15-07-2025

ABSTRACT

Raltegravir, an integrase strand transfer inhibitor (INSTI), is the first FDA-approved drug in its class for HIV therapy.Due to its clinical significance, a variety of analytical methods have been developed for its quantification in pharmaceutical formulations and biological matrices. This review comprehensively compiles 30+ analytical techniques including UV spectrophotometry,RP-HPLC, HPTLC, UPLC, LC-MS/MS, and electrochemical methods, evaluating their sensitivity, specificity, and validation status.The aim is to guide researchers in selecting suitable methods for routine, pharmacokinetic,and stability analysis of Raltegravir.

Keywords: Raltegravir, Analytical Techniques, Method Validation, HPLC, LC-MS/MS.

I. INTRODUCTION

Raltegravir is an antiretroviral drug approved by the US FDA in 2007 for the treatment of HIV-1 infection. It works by inhibiting the HIV integrase enzyme, preventing viral DNA from integrating into the host genome. Chemically known asN-[(4-fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-(1-methylethyl)-6-oxo-4pyrimidinyl]-1,4-dihydro-1,3,5-triazine-2-carboxamide (figure-1). Raltegravir is available in tablet and chewable forms. Given its global use in HAART, robust analytical methods are needed for the detection and quantification of Raltegravir in pharmaceuticals and biological fluids. (1)

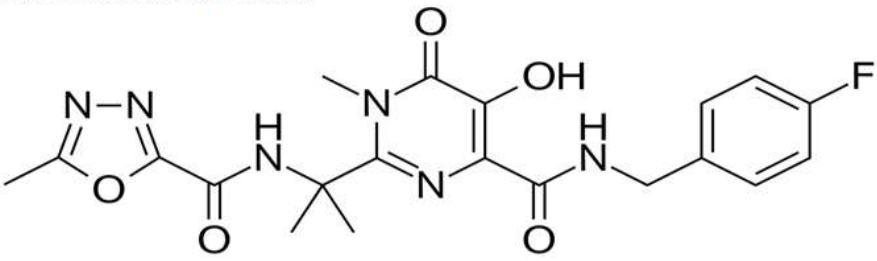

Figure 1 structure of Raltegravir

II. ANALYTICAL METHODS FOR RALTEGRAVIR

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

Spectrophotometric Methods

UV Spectrophotometric method has been developed and validated for estimation of Raltegravir in bulk and tablet dosage form. In this method Raltegravir shows λmax at 290nm using 0.1N NaOH as a solvent and calibration graphs were plotted over the concentrations ranging from 10 to 60 µg/mL of Raltegravir with correlation coefficient 0.999. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.311 µg/mL and 0.941 µg/mL respectively by simple UV spectroscopy (1).

The methods utilized a solvent system of methanol:water (50:50)**,** with absorbance measured at λmax 328 nm and AUC between 323–333 nm. Both methods obeyed Beer's law in the range of 3–55 µg/mL with correlation coefficients >0.996. The

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

methods were validated as per ICH Q2(R1) guidelines, demonstrating good accuracy (recovery 99.36–102.31%)**,** precision (RSD <2%), and robustnessacross varying wavelengths and solvent compositions. The limit of detection (LOD) and limit of quantification (LOQ) for the absorption maxima method were 0.91 and 2.77 µg/mL, and for the AUC method were 1.18 and 3.60 µg/mL, respectively. The assay of marketed tablets (Isentress 400 mg) showed results of 100.58% and 99.69% by absorption maxima and AUC methods, respectively (2).

The analysis was carried out using a solvent system comprising methanol and phosphate buffer (pH 3.0) in the ratio of 55:45 v/v, with absorbance measured at 219 nm**.** The method obeyed Beer's Law in the linearity range of 3–15 µg/mL with a correlation coefficient (r^2) of 0.999, indicating excellent linearity. The limit of detection (LOD) andlimit of quantification (LOQ) were found to be 0.0959 µg/mL and 0.290 µg/mL, respectively. The method demonstrated high accuracy with a mean recovery of 99.96%**,** and %RSD < 2, confirming precision and reliability (3).

The chromatographic separation was achieved using a Symmetry C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 3.0) and methanol in a ratio of 45:55 v/v,at a flow rate of 0.6 mL/min. The detection wavelength was set at 218 nm, and the retention time was approximately 4.35 minutes. The method showed excellent linearity over the concentration range of 10–50 µg/mL, with a correlation coefficient (r^2) of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.027 µg/mL and 0.09 µg/mL, respectively. Accuracy was confirmed through recovery studies, yielding results between 98% and 101.6%, and %RSD values below 2% confirmed the method's precision (3).

UV spectrophotometric methods for the estimation of Raltegravir in bulk and pharmaceutical dosage forms. The techniques included zero-order (D^0), first-order derivative (D^1), and difference spectrophotometry. In the D^0 method, absorbance was measured at 325 nm, and the drug showed linearity over the concentration range of 1–150 µg/mL. The D^1 method used a peak-to-peak measurement between 341 and 377 nm, with a linear range of 10–150 µg/mL, while the difference method measured absorbance between pH 3.0 and pH 7.4 buffers at 325 nm, also showing linearity in the 10–150 µg/mL range. All methods were validated according to ICH Q2(R1) guidelines, confirming their accuracy, precision, linearity, and reproducibility(4).

RP-HPLC methods

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software,RP-HPLC method for the estimation of Raltegravir in bulk and tablet dosage forms. Chromatographic separation was achieved using a Develosil ODS HG-5 RP C18 column (15 cm × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer (pH 3.0) and methanol in a 30:70 ratio, at a flow rate of 1.0 mL/min. Detection was carried out at 246 nm, and the retention time was approximately 3.8 minutes. The method showed linearity in the range of 20–70 µg/mL with a correlation coefficient (r^2) of 0.995. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.06 and 0.36 µg/mL, respectively. Accuracy was confirmed with mean recovery values between 99.94% and 101.20%, and precision was validated with %RSD values below 2%. The method was also robust and rugged under varied analytical conditions (5).

The chromatographic separation was carried out using a C18 column with a mobile phase composed of acetonitrile and phosphate buffer (pH 6.8) in an optimized ratio. The flow rate was maintained at 1.0 mL/min, and detection was performed at 210 nm. The method showed linearity in the concentration range of 10–50 µg/mL with a correlation coefficient greater than 0.999. Validation was done according to ICH guidelines, confirming its accuracy, precision, specificity, and robustness. The retention time of Raltegravir was approximately 3.4 minutes (6).

Chromatographic separation was achieved using a Symmetry C8 column (150 mm × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer (pH 2.5) and acetonitrile in a 40:60 ratio. The flow rate was set at 0.6 mL/min, and detection was performed at 247 nm. The method demonstrated linearity over the concentration range of 5–25 µg/mL with a correlation coefficient of 0.999. The retention time was 2.881 minutes. The method was validated as per ICH guidelines, showing good accuracy with recoveries between 99.36% and 101.85%, and precision with %RSD less than 2%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.006 µg/mL and 0.020 µg/mL, respectively (7).

A validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of raltegravir in combination with

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

lamivudine in solid dosage forms. The chromatographic separation was achieved using an Inertsil ODS C18 column (4.6 × 150 mm, 5 µm) with a mobile phase composed of 0.1% orthophosphoric acid and acetonitrile in a 50:50 (v/v) ratio, at a flow rate of 0.9 mL/min. Detection was carried out at 242 nm, and the retention time for raltegravir was approximately 4.34 minutes. The method exhibited good linearity in the concentration range of 30–150 µg/mL, with a correlation coefficient of 0.999. It was found to be precise, accurate, and specific, with %RSD values below 2% and a mean recovery of 99.89% (8).

A rapid and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantification of Raltegravir potassium in bulk and pharmaceutical dosage forms. The chromatographic separation was performed on a Shim-pack C18 column (150 × 4.6 mm, 5 µm) using a mobile phase consisting of acetonitrile and 0.05 M ammonium acetate buffer (pH 4 adjusted with glacial acetic acid) in a 1:1 ratio, at a flow rate of 0.8 mL/min. Detection was carried out at 271 nm, and the retention time of Raltegravir was approximately 4.31 minutes. The method showed excellent linearity in the range of 10–50 µg/mL with a correlation coefficient greater than 0.9999. It demonstrated good precision with %RSD values below 2%, and high accuracy with recovery between 100–102%. The method was also sensitive, with limits of detection and quantification found to be 0.104 µg/mL and 0.315 µg/mL, respectively. Robustness and specificity tests confirmed the method's reliability for routine analysis of Raltegravir in pharmaceutical quality control (9).

A simple, precise, and cost-effective UV spectrophotometric method was developed and validated for the quantitative estimation of raltegravir potassium in bulk and laboratory-formulated tablets. The method involved preparing a standard solution of the drug in water, with absorption maxima observed at 331.6 nm. It showed excellent linearity over the concentration range of 1–100 µg/mL, with a correlation coefficient of 0.9999. The method demonstrated high precision with intra-day and inter-day relative standard deviation values below 2%, and the mean recovery values ranged from 99.9% to 100.3%, indicating good accuracy (10).

A rigorous RP-HPLC method was devised and validated for the simultaneous determination of raltegravir potassium and its related impurities in bulk drug and tablet formulations. Utilizing a Shim-pack C18 column (150 × 4.6 mm, 5 µm) with an isocratic mobile phase of 50:50 acetonitrile and 0.05 M ammonium acetate buffer (pH 4.0), the method operated at a flow rate of 0.8 mL/min and detected analytes at 271 nm. Raltegravir exhibited a retention time around 4.31 minutes. Validation per ICH guidelines demonstrated excellent linearity (10–50 µg/mL, $r^2 > 0.9999$), precision (%RSD ≈ 1.6%), and accuracy (recovery 100–102%). Sensitivity was notable, with limits of detection and quantitation at 0.104 µg/mL and 0.315 µg/mL, respectively. Specificity was confirmed—no interference from excipients or degradation products at the drug's retention time. Additionally, system suitability parameters (e.g., theoretical plates, tailing factor) met acceptance criteria, and robustness testing showed stable performance despite minor variations in conditions. The method's short run time (~4.3 min), high sensitivity, and compatibility with LC–MS/MS make it well suited for routine quality control and impurity profiling in Raltegravir production (11).

An HPLC method with photodiode array (PDA) detection was developed for the simultaneous quantification of Raltegravir in human plasma, along with other antiretroviral agents. Raltegravir was extracted from 500 µL plasma samples using solid-phase extraction and analyzed on a C18 column with a gradient mobile phase of acetonitrile and phosphate buffer. The method demonstrated excellent linearity for Raltegravir across therapeutic concentrations, with a correlation coefficient above 0.998. It exhibited good precision (intra-day <3.7%, inter-day <6.4%) and accuracy (mean deviation <±1.2%). The extraction recovery for Raltegravir was between 75–83%. This validated method is suitable for therapeutic drug monitoring and pharmacokinetic studies of Raltegravir in clinical settings (12).

An HPLC-PDA method was developed and validated for the simultaneous quantification of raltegravir along with etravirine and 11 other antiretroviral agents in human plasma. Raltegravir was extracted from plasma samples using solid-phase extraction and separated on a reverse-phase C18 column with a gradient mobile phase of acetonitrile and phosphate buffer. Detection was performed using a photodiode array detector. The method demonstrated excellent linearity for raltegravir across therapeutic concentrations ($r^2 > 0.998$), with intra- and inter-day precision below 6.4% and accuracy within ±1.2%. The extraction recovery for raltegravir ranged between 75–83%. This validated assay proved suitable for therapeutic

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

drug monitoring and pharmacokinetic studies in HIV-infected patients (12).

HPLC-UV method for the simultaneous quantification of Raltegravir and Maraviroc in human plasma. Raltegravir was extracted via protein precipitation from plasma, separated on a reversed-phase C18 column under gradient elution, and detected using a UV detector. The assay demonstrated robust linearity ($r^2 > 0.998$) across therapeutic concentration ranges, with acceptable precision (intra- and inter-day variability below ~6%) and good extraction efficiency. This validated method offers a cost-effective and practical approach for therapeutic drug monitoring of raltegravir in clinical settings (13).

A highly sensitive HPLC–MS/MS assay was developed using liquid–liquid extraction and tandem mass spectrometric detection for quantifying Raltegravir in human plasma. The method demonstrated excellent linearity across 1–3,000 µg/mL ($r^2 \approx 0.9992$). Precision ranged from 0.6% to 3.0%, and accuracy fell within 96.5–104.3%. The assay is robust, precise, and sensitive, with successful external proficiency validation, making it well suited for therapeutic drug monitoring and pharmacokinetic studies (14).

Two complementary chromatographic methods—RP-HPLC and HPTLC—for quantifying raltegravir potassium in tablet dosage forms. The RP-HPLC method utilized a C18 column with an acetonitrile–phosphate buffer mobile phase under isocratic conditions, achieving clear separation and a retention time around 4 minutes. The method exhibited excellent linearity ($r^2 > 0.999$) across a defined concentration range, high precision (RSD < 2%), and accurate recovery (~100%). In parallel, the HPTLC technique employed silica gel plates with a methanol–water–acetonitrile mobile phase, enabling robust calibration ($r^2 > 0.999$), precision (RSD < 2%), and recovery (98–102%). Both methods demonstrated specificity, stability, and suitability for routine quality control of Raltegravir potassium in solid formulations, offering versatile, validated tools for pharmaceutical analysis (15).

A highly sensitive LC–MS/MS assay was developed for simultaneous quantification of raltegravir (alongside maraviroc, darunavir, and etravirine) in human plasma. Raltegravir was extracted via single-step protein precipitation, followed by gradient separation on a Waters Atlantis dC18 column and detection by tandem mass spectrometry with electrospray ionization. The method demonstrated excellent linearity over a broad therapeutic range (12.5–5,000 ng/mL), with an extraction recovery exceeding 91%, and inter-day precision within 5.1–9.8% CV. Accuracy remained within –3.3 to +5.1% of nominal values, and the assay enabled simultaneous detection of Raltegravir-glucuronide. This validated, multiplex LC–MS/MS method is well suited for therapeutic drug monitoring and pharmacokinetic assessment of Raltegravir (16).

A sensitive and specific HPLC method with fluorescence detection was developed for quantifying raltegravir (MK-0518) in human plasma. Plasma samples (500 µL) were subjected to liquid–liquid extraction using a hexane/methylene chloride mixture at pH 4.0, with delavirdine as the internal standard. Separation was achieved on a Symmetry Shield RP18 column under a gradient elution of acetonitrile and 0.01% triethylamine buffer (pH 3.0) at a flow rate of 1 mL/min. Fluorimetric detection was set at 299 nm excitation and 396 nm emission. Raltegravir eluted at ~6.4 minutes, and calibration curves were linear from 5 to 1,000 ng/mL. The method displayed high accuracy (98.3–101.0%) and precision (≤ 6.3%), with no interference from co-administered antiretrovirals. This fluorescence-based assay is well-suited for therapeutic monitoring in clinical settings (17).

A reverse-phase HPLC method was developed and validated for the simultaneous estimation of raltegravir and lamivudine in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using an Inertsil ODS 3V C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0) in a 45:55 v/v ratio, at a flow rate of 1.0 mL/min. Detection was performed using a photodiode array (PDA) detector at 275 nm. The method exhibited good linearity in the range of 150–450 µg/mL for raltegravir and 50–150 µg/mL for lamivudine, indicating its suitability for routine quality control analysis (18).

An RP-HPLC method was developed for the simultaneous estimation of lamivudine and raltegravir in bulk and tablet dosage forms. The separation was achieved on a Symmetry C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of orthophosphoric acid and acetonitrile (40:60 v/v), with pH adjusted to 2.45 using triethylamine. The flow rate was maintained at 1.0 mL/min, and detection was carried out using a PDA detector at 260 nm. The retention times for lamivudine and raltegravir were 2.355 and 3.400 minutes, respectively. The method was found to be suitable for routine analysis in combined dosage forms (19).

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

A rapid and specific RP-HPLC method was developed and validated for quantifying raltegravir in human plasma. After liquid–liquid extraction using methyl-tert-butyl ether, samples were analyzed on a Phenomenex C18 column (250 × 4.6 mm, 5 µm) using a 60:40 v/v mixture of 10 mM phosphate buffer (pH 3.5) and acetonitrile. Detection was achieved via UV at 268 nm. Linearity was established across a concentration range of 40–4,003.9 µg/mL, with a limit of detection at 40 µg/mL. The method showed good recovery (78.7%) and precision (CV 1.5%). Intra-day accuracy ranged from 96.8–102.2% (precision 3.5–5.8%), and inter-day accuracy ranged from 94.3–103.5% (precision 2.7–7.7%). Importantly, plasma interferences were well resolved, and the assay met FDA validation guidelines, making it suitable for therapeutic monitoring and pharmacokinetic studies (20).

A comprehensive study employed eight spectrophotometric techniques—including zero-order, first-order derivative, and difference spectroscopy—to quantify Raltegravir in film-coated tablets. Using buffers such as borate (pH 9), hydrochloric acid, and sodium acetate, the methods achieved linearity across various ranges: 1–150 µg/mL (zero-order), 10–150 µg/mL (first-order derivative), and 10–150 µg/mL (difference spectroscopy). All techniques were validated per ICH guidelines, demonstrating high precision and accuracy. This set of methods offers simple, cost-effective, and reliable alternatives for routine analysis of raltegravir in pharmaceutical formulations (21).

A robust and sensitive RP-HPLC method was developed and optimized using a Design of Experiment (DOE) approach for the simultaneous estimation of raltegravir and lamivudine in binary mixtures. Separation was achieved on a Phenomenex Luna C18 column (150 × 4.6 mm, 5 µm) with a mobile phase comprising 75% methanol, 15% acetonitrile, and 10% 0.05 mM phosphate buffer (pH 3.0), at a flow rate of 1.2 mL/min. Detection was performed at 254 nm. The retention times were 3.13 ± 0.07 minutes for lamivudine and 7.27 ± 0.01 minutes for raltegravir. The method demonstrated linearity in the range of 10–100 µg/mL for lamivudine and 5–30 µg/mL for raltegravir, with LODs of 1.04 µg/mL and 0.36 µg/mL, respectively. The method showed good accuracy (96.5–102.5% recovery), precision (%RSD < 2%), and robustness, with no interference from excipients, making it suitable for routine analysis in combination drug formulations (22).

A selective HPLC–UV assay for simultaneous quantification of nine antiretroviral drugs—including Raltegravir—in human plasma. Plasma samples (500 µL) underwent solid-phase extraction. Chromatographic separation was achieved on a C18 reverse-phase column with a gradient of acetonitrile and sodium acetate buffer over a 25-minute run. The method demonstrated excellent linearity for Raltegravir within its therapeutic range ($r^2 > 0.99$), with intra- and inter-day precisions under 15% RSD, and accuracy deviations below 15%. Extraction recovery for Raltegravir fell between 80–120%. Overall, the assay proved sensitive, reliable, and well-suited for therapeutic drug monitoring and pharmacokinetic studies (23).

This study applied the Quality by Design (QbD) approach to develop a robust, stability-indicating HPLC method for the analysis of raltegravir and its degradants in the presence of excipients. The method development was guided by defining the Analytical Target Profile (ATP), performing risk assessments, and using Design of Experiments (DoE) to understand the effects of method variables. A full factorial design was employed to evaluate the impact of flow rate, column temperature, organic solvent ratio, and buffer pH on chromatographic performance. The method was optimized using an Inertsil C18 column, with statistical analysis identifying organic solvent content as a critical factor interacting with flow rate and pH to achieve the required USP resolution (not less than 1.8). The final method was validated per ICH guidelines and demonstrated acceptable accuracy, precision, linearity, specificity, and robustness (24).

A simple, precise, and robust RP-HPLC-UV method was developed and validated for the quantitative estimation of Raltegravir in bulk and pharmaceutical dosage forms. Chromatographic separation was performed on an Agilent C18 column (100 × 2.5 mm, 3 µm) using an isocratic mobile phase of acetonitrile and water (80:20, v/v) at a flow rate of 0.8 mL/min with detection at 240 nm. Raltegravir showed a retention time of 1.38 minutes. The method exhibited excellent linearity in the concentration range of 25–200 µg/mL (r^2 = 0.9997), with mean recovery between 99.7–100.7%. Precision studies showed %RSD < 2% for both intra-day and inter-day analysis. System suitability parameters such as theoretical plates (3258) and tailing factor (0.6) were within acceptable limits. The method also demonstrated good robustness and ruggedness under deliberate variations in conditions. The

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

validated method was successfully applied to the assay of marketed tablet formulations and is suitable for routine quality control and stability studies(25).

A simultaneous RP-HPLC method was developed and validated for the quantification of both lamivudine and raltegravir in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column, with a mobile phase composed of a phosphate buffer (pH 6.8) and acetonitrile (typically around 60:40 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 260 nm. Raltegravir and lamivudine were well resolved, with sharp and symmetric peaks. The method was validated to meet ICH guidelines, showing excellent linearity, high precision (%RSD < 2%), and strong accuracy (recovery 98–102%), making it suitable for routine quality control of fixed-dose combinations (26).

A rapid and reliable HPLC-UV method was developed and validated for the quantification of Raltegravir in human plasma using solid-phase extraction (SPE). The assay was optimized for high selectivity and minimal interference from co-administered drugs. Chromatographic separation was performed on a C18 column with UV detection, and the method demonstrated linearity over the range of 20–10,000 µg/mL. The average extraction recovery was 90% for both Raltegravir and the internal standard (diazepam). Intra- and inter-day precision ranged from 1.4% to 7.9%, and accuracy ranged from 97.5% to 104.4%. This method was noted as the first to use UV detection with SPE for raltegravir and is suitable for routine therapeutic drug monitoring and pharmacokinetic analysis (27).

A validated, stability-indicating RP-HPLC method with UV detection (210 nm) was developed for the analysis of Rasagiline mesylate in pharmaceutical dosage forms and its forced degradation products. Chromatographic separation was achieved on a Hypersil BDS C18 column using an isocratic mobile phase of phosphate buffer and acetonitrile (45:55, v/v) at a flow rate of 1.2 mL/min. The method showed excellent linearity over the range of 25–150% of the specification limit (r^2 = 0.999), with high precision (%RSD = 0.72) and accuracy (recoveries between 99.4%–100.1%). Forced degradation studies were conducted under acidic, basic, oxidative, thermal, and photolytic conditions. Rasagiline showed significant degradation under all stress conditions except oxidation. The degradation products were identified and characterized using LC-MS/MS, revealing pathways including hydrolysis of carbamate and urea linkages. The method was validated according to ICH guidelines and was proven to be specific, robust, and suitable for routine quality control and stability analysis (28).

A stability-indicating HPLC method was developed to assess the degradation behavior of Raltegravir (RAL) under ICH-recommended stress conditions, including hydrolytic (acidic and alkaline), oxidative, photolytic, and thermal environments. Significant degradation was observed under alkaline hydrolysis, with major degradation products proposed. The method employed silica gel GF254 plates and a mobile phase of toluene, ethyl acetate, methanol, and acetic acid (8:1:1:0.1, v/v/v/v), with detection at 299 nm. Linearity was observed in the range of 200–700 µg/spot (r = 0.9923), and the method showed good accuracy, precision, and specificity. LOD and LOQ were 33.99 µg/spot and 103.01 µg/spot, respectively. The degradation followed zero-order kinetics, with an activation energy of 5.99 kcal/mol (29).

A rapid, robust, and stability-indicating UPLC method was developed and validated for the simultaneous estimation of raltegravir (RAL) and lamivudine (LMV) in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved within 4 minutes using a BEH Shield RP18 column (2.1 mm × 100 mm, 1.7 µm) with an isocratic mobile phase comprising potassium dihydrogen orthophosphate buffer (pH 3, adjusted with orthophosphoric acid) and methanol (30:70, v/v) at a flow rate of 0.230 mL/min. Detection was carried out at 254 nm using a PDA detector (30).

III. CONCLUSION

Raltegravir, a pioneering HIV integrase inhibitor, has significantly advanced antiretroviral therapy through its potent efficacy and favorable safety profile. With its widespread clinical application, the development of precise, validated analytical methods is crucial for ensuring accurate quantification in both pharmaceutical formulations and biological matrices. This review has outlined a broad spectrum of analytical techniques—including UV spectrophotometry, RP-HPLC, HPTLC, UPLC, LC-MS/MS, and HPLC-PDA—each offering distinct advantages in terms of sensitivity, specificity, speed, and applicability. RP-HPLC continues to be the most frequently employed due to its balance of precision, simplicity, and cost-efficiency. Advanced techniques like LC-MS/MS provide superior sensitivity, making them highly suitable for pharmacokinetic and therapeutic drug

monitoring studies. The availability and continual development of these methods reinforce quality control, enhance regulatory compliance, and support optimal therapeutic outcomes in the management of HIV with Raltegravir.

Acknowledgements
The authors are grateful to the Principal, GokarajuRangaraju College of Pharmacy and GokarajuRangaraju Educational Society for providing necessary infrastructure facilities.

REFERENCES

[1]. **Siddartha B, Sudheer Babu I.** UV–Spectrophotometric Method for Estimation of Raltegravir in Bulk and Tablet Dosage Form. Int J Pharm Chem Biol Sci. 2014;4(4):807–811.

[2]. **Kore PP, Gamepatil MM, Nimje HM, Baheti KG.** Spectrophotometric estimation of Raltegravir potassium in tablets. Indian J Pharm Sci. 2014;76(6):557–559.

[3]. **Sudha T, Raghupathi T.** Reverse phase–high performance liquid chromatography and ultra violet spectrophotometric method for the estimation of Raltegravir potassium in bulk and in tablet dosage form. Glob J Med Res. 2011;11(2):1–8.

[4]. **Annapurna MM, Seetharami Reddy B, Nagaraju B.** New validated UV spectrophotometric methods for the estimation of Raltegravir in bulk and pharmaceutical dosage forms. Asian J Pharm. 2018;12(4):1428–1434.

[5]. **Shirisha B, Srinivasa Rao A, Sharone Aneeta B, Sumanth Reddy G, Azhaniddin M, Basanth Reddy B, Arun T.** Method development and validation of Raltegravir by RP-HPLC method. Int J Pharm Anal Res. 2019;8(3):320–328.

[6]. **Rambabu K, Balamurali KK, Sambasiva R.** New RP-HPLC method development and validation for analysis of antiviral drug Raltegravir. Int J Res Pharm Biomed Sci. 2011;2(1):132–135.

[7]. **Lakshmana Rao A, Raghu Ram MS.** Validated reverse phase HPLC method for determination of Raltegravir in pharmaceutical preparations. Int J Res Pharm Chem. 2012;2(1):217–221.

[8]. Tejaswi JKD, Rajan RG. RP-HPLC method development and validation for simultaneous estimation and forced degradation studies of lamivudine and raltegravir in solid dosage form. Int J App Pharm. 2018;10(6):242–248. https://doi.org/10.22159/ijap.2018v10i6.28027

[9]. Patel S, Nagappan KV, Reddy GS, Kowmudi G. Quantitative Reverse-Phase High-Performance Liquid Chromatographic Method for the Quantification of Raltegravir Potassium in Bulk and Dosage Forms. J YoungPharm.2019;11(3):274–278. https://doi.org/10.5530/jyp.2019.11.55

[10]. Bhavar GB, Pekamwar SS, Aher KB, Chaudhari SR. Simple spectrophotometric method for estimation of raltegravir potassium in bulk and pharmaceutical formulations. J Appl Pharm Sci. 2013;3(10):147–150. https://doi.org/10.7324/JAPS.2013.31026

[11]. Balaji M, Apparao KMC, Ramakrishna K, Srinivasarao V. Development and validation of RP-HPLC method for determination of raltegravir and its impurities in bulk drug and dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;5(3):187–196.

[12]. D'Avolio A, Baietto L, Siccardi M, Sciandra M, Simiele M, Oddone V, Bonora S, Di Perri G. An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the NNRTI etravirine and 11 other antiretroviral agents in plasma of HIV-infected patients. Ther Drug Monit. 2008;30(6):662–669.

[13]. Notari S, Trezza G, Rossi F, Decimo D. Simultaneous determination of maraviroc and raltegravir in human plasma by HPLC-UV. IUBMB Life. 2009;61(4):470–475.

[14]. Long MC, Bennetto-Hood C, Acosta EP. A sensitive HPLC–MS–MS method for the determination of raltegravir in human plasma. J Chromatogr B. 2008;867(2):165–171.

[15]. Sudha T, Shanmugasundram S. Development and validation of RP-HPLC and HPTLC chromatographic methods for the quantitative estimation of raltegravir potassium in pharmaceutical dosage forms. Res J Pharm Technol. 2011;4(11):1746–1750.

[16]. Decosterd LA, Fayet A, Béguin A, Zanolari B, Cruchon S, Guignard N, Telenti A, Cavassini M, Günthard HF, Buclin T, Biollaz J, Rochat B. A LC-tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, Maraviroc,

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 183-190 www.ijprajournal.com ISSN: 2456-4494

Darunavir and Etravirine. J Chromatogr B. 2009;877(11-12):1057–1069.

[17]. Poirier JM, Robidou P, Jaillon P. Quantification of the HIV-integrase inhibitor raltegravir (MK-0518) in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2008;867(2):277–281.

[18]. Junapudi S, Nagaraju P, Ganesh K, Nagesh M. Method development and validation for simultaneous estimation of raltegravir and lamivudine by using RP-HPLC in bulk and pharmaceutical dosage form. Inventi Rapid: Pharm Analysis & Quality Assurance. 2018;1–5.

[19]. Priyanka B, Sahithi K, Srihitha G, Vyshnavi G, Sravanthi Bijjiga. Analytical method development and validation for the simultaneous estimation of lamivudine and raltegravir by RP-HPLC method. Pharma Research Library.

[20]. Murthy PS, Panakala Rao VV, Rama Babu G. Development and validation of RP-HPLC method for the determination of raltegravir in human plasma. J Applicable Chem. 2017;6(4):559–567.

[21]. Mukthinuthalapati MA, Gunnam RT, Ponnekanti KPS, Boni C. Determination of Raltegravir by derivative and difference spectrophotometric techniques in film coated tablets. Res J Pharm Technol. 2017;10(4):991–997. doi:10.5958/0974-360X.2017.00180.9

[22]. Singh VD, Daharwal SJ. Optimization of RP-HPLC method for simultaneous estimation of lamivudine and raltegravir in binary mixture by using design of experiment. Eurasian J Anal Chem. 2017;12(3):179–195. doi:10.12973/ejac.2017.00162a

[23]. Charbe N, Baldelli S, Cozzi V, Castoldi S, Cattaneo D, Clementi E, et al. Development of an HPLC–UV assay method for the simultaneous quantification of nine antiretroviral agents in the plasma of HIV-infected patients. J Pharm Anal. 2016;6(6):396–403.

[24]. Kancherla P, Dhulipalli SB, Alegte P, JVLN SR. Development and validation of chromatographic method for related substances of raltegravir in raltegravir tablets using quality by design (QbD) approach. Int J Pharm Sci Rev Res. 2016;40(1):259–265.

[25]. Vijaya Sri K, Ravinderreddy S, Suresh K. Rapid RP-HPLC method development and validation for analysis of raltegravir in bulk and pharmaceutical dosage form. Asian J Res Chem. 2015;8(5):335–339. doi:10.5958/0974-4150.2015.00055.3

[26]. Lavanya K, Koteswararao N, Srinivasa Rao V. 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. 2017;5(1):49–59.

[27]. Rezk NL, White N, Kashuba AD. An accurate and precise high-performance liquid chromatography method for the rapid quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction. Anal Chim Acta. 2008;628:204–213.

[28]. Bhavyasri K, Balaram M, Nageswarao V, Rambabu R, Sasikiran Goud DE, Ajitha M. Development and validation of forced degradation studies of Raltegravir using RP-HPLC and characterization of degradants by LC-MS/MS. J Pharm Sci Res. 2015;7(9):685–689.

[29]. Patidar S, Vengurlekar S, Jain SK. Development and validation of analytical method of raltegravir, an antiviral drug. Asian J Pharm Anal. 2024;14(1):21–25. doi:10.52711/2231-5675.2024.00005

[30]. Konari SN, Jacob JT. Stability indicating validated UPLC technique for the simultaneous analysis of raltegravir and lamivudine in pharmaceutical dosage forms. HIV AIDS Rev. 2016;15(4):161–169.