ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 4, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

GREEN ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF RALTEGRAVIR IN DRUG AND PHARMACEUTICALS

SUMITHRA M*, ARCHANA K

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology, and Advanced Studies, Chennai, Tamil Nadu, India.
***Corresponding author: Sumithra M; Email: hodpchemistry@vistas.ac.in**

Received: 08 February 2025, Revised and Accepted: 21 March 2025

ABSTRACT

Objectives: The objective of this study was to develop and validate a green solvent-assisted chromatographic method for the detection of raltegravir in bulk and pharmaceutical formulations. The study aimed to ensure an environmentally sustainable approach while maintaining precision, accuracy, and regulatory compliance.

Methods: A chromatographic method was developed using ethanol and aqueous amine buffer (85:15%v/v) as the mobile phase at pH 4.0. The method was optimized with a flow rate of 1.0 mL/min, and the compound was detected at 247 nm. Validation was conducted following the International Council for Harmonization (ICH) guidelines, assessing key parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), and forced degradation studies. The greenness of the method was evaluated using various green chemistry assessment tools, including the Analytical GREEnness Approach (AGREE), green analytical procedure index (GAPI), Complex GAPI, red analytical performance index, blue applicability grade index, and Red Green Blue (RGB) assessment models.

Results: The developed method achieved a retention time of 5.46 min and exhibited a strong linear relationship with a correlation coefficient (r^2) of 0.999 within the concentration range of 3.0–15 µg/mL. The LOD and LOQ values were within acceptable criteria, demonstrating the method's sensitivity. The greenness assessment revealed that the AGREE score was close to 1, while hexagonal charts with green centers confirmed the eco-friendliness of the method in comparison to reported conventional techniques. Forced degradation studies indicated acceptable degradation levels ranging from 5% to 20%, in compliance with ICH Q1B guidelines, ensuring the method's robustness and stability-indicating capability.

Conclusion: The proposed green solvent-assisted chromatographic method provides an eco-friendly, precise, and accurate approach for the analysis of raltegravir in bulk and pharmaceutical formulations. The method aligns with green analytical chemistry principles and regulatory guidelines, offering a sustainable alternative to conventional chromatographic techniques.

Keywords: Raltegravir, Green method, Ethanol, High-performance liquid chromatography, GAC.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i4.54179. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Green analytical chemistry principles aim to reduce the consumption of solvents, energy resources, and environmentally hazardous wastes [1-3]. Ethanol is classified as a green solvent according to the Environmental Protection Agency (EPA) [4-7]. It is not bio-accumulative as per the World Health Organization, Agency for Toxic Substances and Disease Registry, EPA, and International Program on Chemical Safety [8,9]. It is non-toxic to aquatic life owing to its low LC_{50} value [10], high water solubility, volatility, and rapid degradation [11-13]. The use of green solvents instead of traditional high performance liquid chromatography (HPLC) solvents affects the environment and analysts in an unbiased manner [14-17]. This study aimed to develop and validate a green solvent-assisted chromatographic method for the determination of raltegravir in drug formulations and to evaluate the greenness of the developed method with six significant greenness evaluator tools, namely Analytical GREEnness Approach (AGREE), green analytical procedure index (GAPI), Complex GAPI, blue applicability grade index (BAGI), red analytical performance index (RAPI), and RGB. All six evaluators incorporated the criteria for sample analysis, waste management, renewable reagents, analyst safety, and the practicability of the method. AGREE is a user-friendly approach with a score of <1 and a green color to assess greenness [18,19]. GAPI and Complex GAPI analyze the analytical procedures with a color-indicated hexagonal pictogram [20-22]. The BAGI and RAPI represent the practicability of the method with a high-score representation. RGB represents white analytical chemistry compared to existing methods [23].

As per the literature review, many studies have been carried out to quantify raltegravir [24,25] in pharmaceutical-dosage forms using traditional HPLC solvents such as acetonitrile, which has a hazardous effect on the environment [26] in many ways, [24,27-35]. However, other antivirals have been estimated using green solvents using different techniques, such as micellar electrokinetic chromatography for oseltamivir [36,37], micellar liquid chromatography for acyclovir [38,39], and eco-friendly methods using green solvents to estimate zidovudine [40]. Raltegravir estimation has so far been reported using the above traditional methods and toxic solvents. To date, no method has been reported in the field of green analytical chemistry.

METHODS

Reagents and apparatus

The proposed method was opted for raltegravir API (99.8% pure) procured from Maithri Drugs, Telangana. The marketed dosage form (Zepdon) of 400 mg was procured from the local market, and the drug sample was authenticated using a melting point of 157°C and infrared analysis. In addition, HPLC water, HPLC Ethanol (≥99.9%) procured from Merck, and triethylamine (99.5%) were obtained from Thermo Fisher Chemicals. The HPLC WATERS Alliance 2695 separation module with a PDA detector and Empower 2 software, pH meter of

Lab India, and Sartorius digital weighing balance were used for our process.

Optimized conditions
A mixture of ethanol (850 mL) and aqueous triethylamine buffer (150 mL) at pH 4.0 was prepared, degassed, and used as the mobile phase. The mobile phase was supplied at 1.0 mL/min flow rate 247 nm used as detection wavelength. An injection volume of 10 µL with a run time of 10 min was maintained in a Hypersil C18 column. The recorded theoretical plates were not <2,000, and the tailing factor was not >2. All chromatographic parameters were within the acceptable range according to the International Council for Harmonization (ICH) Q2 A&B guidelines.

Linearity
A series of diluted stock solutions with concentration of 3–15 µg/mL were injected into the HPLC system. The calibration curve was plotted between the concentration and peak area to assess linearity. The regression coefficient, which intercepts according to the ICH guidelines, was obtained.

Preparation of stock standard and sample solution
An initial stock solution was prepared by dissolving 100 mg of raltegravir in 70 mL of diluent in a 100 mL volumetric flask, followed by sonication to ensure complete dissolution. This solution was brought up to volume with the same diluent. The filtered stock solution (0.7 mL) was diluted to 100 mL with a diluent to obtain the desired concentration.

Preparation of sample solution
Ten tablets were weighed, crushed, and powdered using a mortar and pestle, and an equivalent weight of 100 mg of the raltegravir formulation was diluted with 70 mL of diluent in a 100 mL volumetric flask, followed by sonication to ensure complete dilution. Furthermore, the volume was increased using the same diluent. After filtration, the stock solution (0.7 mL) was diluted to 100 mL with a diluent to obtain a final concentration of 7 µg/mL.

System suitability
After optimization of chromatographic conditions for analysis, 10 µL of the standard solution was injected 5 times into the HPLC, and validation parameters such as retention time, peak area, peak height, tailing factor, and theoretical plates were examined for system suitability.

Validation of the method
The developed method was evaluated according to ICH Q2 A&B guidelines to ensure reproducibility.

Evaluation of developed method by green metric tools
Using greenness evaluator platforms AGREE, GAPI, Complex GAPI, RAPI, BAGI, and RGB, the developed method was verified to understand the greenness of the method based on the 12 Green Analytical Chemistry principles and white analytical chemistry.

Stress testing studies
Forced degradation studies were carried out under hydrolysis (acid and base), peroxide, thermal, and sunlight conditions. Ten tablets were weighed, crushed, and powdered, and an equivalent weight of 100 mg of raltegravir was transferred to a 100 mL volumetric flask and diluted to the mark. The above solution was used as the stock solution.

RESULTS

Extensive trials have led to the development of optimized chromatographic methods for antiviral drug analysis. The mobile phase was comprised of a mixture of ethanol (850 mL) and aqueous triethylamine buffer (150 mL) adjusted to pH 4.0, degassed, and maintained at a flow rate of 1.0 mL/min. Detection was performed at 247 nm using a Hypersil C18 column with a 10 µL injection volume and a runtime of 10 min. The system suitability parameters adhered to ICH Q2 A and B guidelines.

Linearity
The linearity was evaluated between 3.0 µg/mL and 15 µg/mL. It is assessed between different criteria of limit of detection (LOD) and the results explore the linear relationship between the parameters with an r^2 value of 0.999 in the acceptable range as per the ICH Q2 A&B guidelines. This plot is shown in Fig. 1.

System suitability
The retention time of the both standard and sample were found to be at 5.46±0.02 min with a tailing factor of 1.62 not more than 2 and theoretical plates of 9,658 not <2,000. The capacity factor of k' was 2.64 with dead and retention times.

Specificity
To precisely quantify raltegravir in the pharmaceutical drug product, the specificity of the method was examined. The chromatograms obtained are shown in Figs. 2 and 3, and the data are tabulated in Table 1.

Recovery studies
Three times, both standard solutions and sample solutions were injected into the chromatographic system for recovery study analysis. The percentage purity was found to be 99.69–100.011%, which is the acceptance criteria for estimating raltegravir in drug products.

Precision
Repeatability and reproducibility
Five replicates of 100% accurate solutions were injected, and the peak areas and RSD% were computed for repeatability analysis. Six replicates of the samples were injected by different analysts under similar conditions in HPLC, and the RSD% was computed for reproducibility analysis. The reproducibility study shows that both analysts obtained consistent results, with RSD% values below 2%, confirming high precision. The p=0.006 indicates that there is no significant difference between the two analysts' measurements, supporting the method's reproducibility. The 95% confidence intervals (CI) for both analysts overlap, further confirming that the variations observed are within acceptable experimental limits. The RSD% of both repeatability and reproducibility was >2, which is within the acceptance criteria. The data are tabulated in Tables 2-4.

Accuracy
The method's accuracy was rigorously evaluated through percent recovery studies at three distinct concentration levels: 50%, 100%, and 150%. The mean recovery was calculated to be 100.43%, with a 95% CI extending from 99.52% to 101.34%. A one-sample t-test was conducted to ascertain whether the recovery values significantly deviated from the expected 100% recovery rate. The resulting t-statistic was 2.047, accompanied by a p=0.005, suggesting that the observed recovery values did not significantly differ from 100% and remained within acceptable parameters. In addition, the effect size, represented by Cohen's d=1.18, underscores the method's strong practical accuracy. The mean recovery values are systematically documented in Table 5.

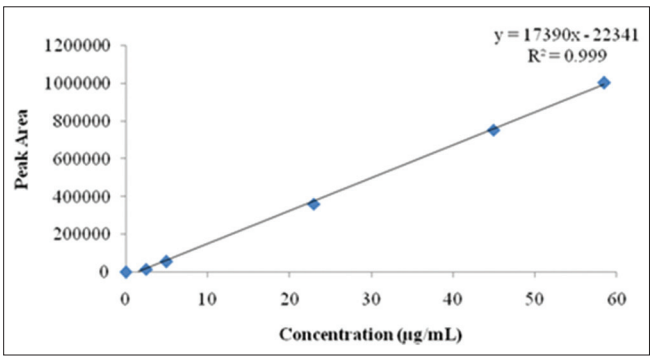

Fig. 1: Linearity graph of raltegravir

Table 1: Results of optimized conditions of raltegravir (standard and sample)

S. No.	Name	RT in min	Area (µV×s)	Height	USP tailing	USP plate count
1	Raltegravir (std)	5.462	1,052,689	75,421	1.62	9,625
2	Raltegravir	5.466	1,068,547	76,584	1.63	9,658

[1]Mean : 1265610, SD: 257.2184, %RSD: 0.020324

Table 2: Results of repeatability for raltegravir[1]

Serial number	Sample name	RT (min)	Area (µV×s)	Height (µV)	USP plate count	USP tailing
1	Raltegravir (7 µg/mL)	5.419	1,265,666	76,231	9,658	1.63
2	Raltegravir	5.405	1,265,895	75,898	9,667	1.62
3	Raltegravir	5.478	1,265,468	75,452	9,652	1.63
4	Raltegravir	5.466	1,265,774	75,468	9,635	1.62
5	Raltegravir	5.466	1,265,245	76,214	9,658	1.63

[2]Mean area: 1265540, SD: 271.5347, %RSD: 0.021456

Table 3: Results of reproducibility for raltegravir by analyst-I[2]

Serial number	Sample name	RT (min)	Area (µV×s)	Height (µV)	USP plate count	USP tailing
1	Raltegravir (7 µg/mL)	5.484	1,265,846	76,985	9,785	1.65
2	Raltegravir	5.493	1,265,254	76,854	9,748	1.64
3	Raltegravir	5.406	1,265,598	76,254	9,786	1.65
4	Raltegravir	5.419	1,265,461	76,859	9,726	1.65
5	Raltegravir	5.446	1,265,236	75,898	9,742	1.64
6	Raltegravir	5.452	1,265,842	76,985	9,785	1.65

Table 4: Results of reproducibility for raltegravir by analyst-II[3]

Serial number	Sample name	RT (min)	Area (µV×s)	Height (µV)	USP plate count	USP tailing
1	Raltegravir (7 µg/mL)	5.493	1,265,545	78,574	9,865	1.65
2	Raltegravir	5.493	1,264,547	78,546	9,854	1.64
3	Raltegravir	5.478	1,265,588	78,452	9,826	1.65
4	Raltegravir	5.466	1,265,542	78,542	9,824	1.65
5	Raltegravir	5.478	1,265,243	78,563	9,863	1.66
6	Raltegravir	5.419	1,265,874	78,632	9,875	1.66

[3]Mean area: 1265390, SD: 458.8784, %RSD: 0.036264

Table 5: Results of accuracy of raltegravir (standard and sample)

%concentration (at specification level)	Area (µV×s)	Amount added (µg/mL)	Amount found (µg/mL)	Percentage recovery	Mean recovery	t-statistic	p
50%	632,729	23	23.154	100.6696	100.4335%	2.047	0.005
100%	1,265,114	45	45.279	100.62			
150%	1,898,868	67.5	67.508	100.011			

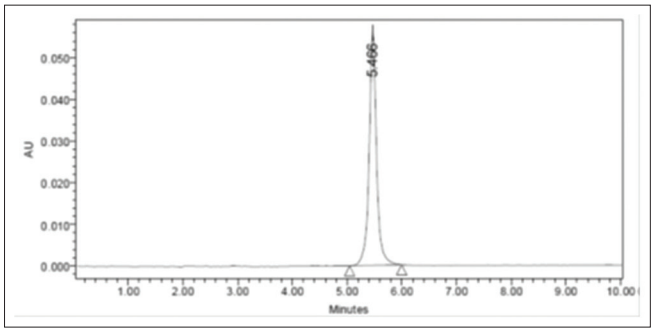

Fig. 2: Optimized chromatogram of raltegravir (std) - 7 µg/mL

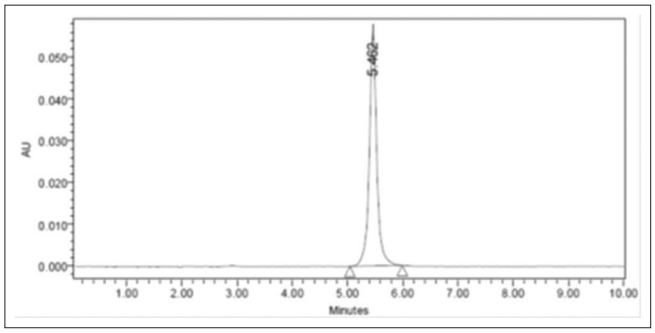

Fig. 3: Optimised chromatogram of raltegravir (sample) - 7 µg/mL

Limit of quantification (LOQ) and LOD

The LOQ and LOD were computed according to the ICH Q2A&B guidelines and were found to be 1.6 µg/mL and 4.8 µg/mL.

Robustness studies

The proposed method was tested for standards and samples with varying flow rates and compositions of organic phases in varying proportions. The method is robust under lower flow conditions and by changing the mobile phase by ±5%. Results are tabulated in Table 6. The robustness data indicate that the method remains reliable under small variations in flow rate and organic phase composition, with a %RSD of 1.54%, which is well within acceptable limits. However, the p=0.022 suggests that the observed variations are statistically significant.

Analytical method greenness and comparison

The environmental friendliness of the developed method was assessed using the AGREE, GAPI, complex GAPI, BAGI, RAPI, and RGB platforms. AGREE provided a score near 1, indicating strong eco-friendliness, whereas GAPI and complex GAPI presented green-centered hexagonal charts that visually highlighted the greenness of the developed method. Figs. 4-6 collectively show the method's high greenness compared to the reported methods, underscoring the reported method's sustainability. The practicability and efficiency of the method are represented using the BAGI and RAPI, as shown in Fig. 7. A comparison of existing methods with the proposed ethanol-based method is presented using the RGB aspect of white analytical chemistry in Fig. 8.

Forced degradation studies

These studies demonstrated drug degradation in comparison with the sample area of the standard. The lowest degradation was observed for peroxide and photolytic acid, and the highest degradation was observed under thermal and alkaline conditions. The percentage of degradation is presented in Table 7 and Fig. 9. Drugs exposed to high temperatures and alkaline conditions are prone to rapid degradation, leading to reduced efficacy and stability. To prevent thermal degradation, pharmaceutical formulations should be stored at controlled temperatures, ideally in cool, dry environments [41]. Heat-sensitive drugs should be refrigerated or stored in climate-controlled facilities to prevent bond cleavage and decomposition [42]. In addition, exposure to direct heat sources such as sunlight or manufacturing processes involving high temperatures should be minimized [43]. In the case of alkaline degradation, which often results from hydrolysis, formulations should be buffered to maintain a stable pH and minimize interaction with strong bases [44]. Using pH-adjusting agents or selecting excipients that resist alkaline hydrolysis can significantly enhance stability [45]. Furthermore, protective coatings or encapsulation techniques can shield drugs from alkaline environments, particularly in liquid formulations. By implementing these precautions, degradation under thermal and alkaline conditions can be effectively minimized, ensuring longer shelf life and improved pharmaceutical stability.

DISCUSSION

The proposed method is a validated green analytical method for the determination of raltegravir in drugs and pharmaceuticals. No methods have been reported using green solvents, as per the extensive literature review. The development of these methods is required to reduce solvent consumption and ensure safety for analysts and the environment. Optimized conditions of the method 'ethanol (850 mL): (150 mL) at pH 4.0 and Hypersil C18 column with a run time of 10 min revealed an acceptable separation with a retention time of 5.462 min. Table 8 demonstrates consistent and reproducible results that comply with the ICHQ2A&B guidelines. The method demonstrated high accuracy, as confirmed by recovery studies conducted at 50%, 100%, and 150% concentration levels,

Table 6: Results of robustness of raltegravir

Serial number	Parameter used for sample analysis	Area (µV×s)	RT (min)	Theoretical plates	Tailing factor
1	Actual flow rate of 1.0 mL/min	1,252,689	5.453	9625	1.62
2	Less flow rate of 0.9 mL/min	1,215,241	5.599	9155	1.54
3	More flow rate of 1.1 mL/min	1,223,654	4.576	9254	1.56
4	More organic phase	1,215,853	3.827	9147	1.54
5	Less organic phase	1,202,514	7.415	9256	1.53

Fig. 4: Raltegravir with acetonitrile and ethanol green metrics using analytical greenness metric approach

Table 7: Results of forced degradation studies of raltegravir

Serial number	Degradation	Sample weight (µg/mL)	Time (days)	Storage conditions (%)	Assay (% w/w); Raltegravir	Degradation
1	Acid (0.1 N HCl)	7 µg/mL of raltegravir	3 h	25°C, 60 RH	93 (7)	Mild
			7	25°C, 60 RH	90 (10)	Mild
			90	40°C, 75 RH	87 (13)	Moderate
2	Alkaline (0.1 N NaOH)	7 µg/mL of raltegravir	3 h	25°C, 60 RH	92 (8)	Mild
			1 week	40°C, 75 RH	85 (15)	Rapid
			3 months	40°C, 75 RH	80 (20)	Significant
3	Peroxide (0.1% H_2O_2)	7 µg/mL of raltegravir	3 h	25°C, 60 RH	94 (6)	Mild
			1 week	40°C, 75 RH	91 (9)	Mild
			3 months	40°C, 75 RH	88 (12)	Moderate
4	Thermal (heat chamber	7 µg/mL of Raltegravir	3 h	60°C	91 (9)	Mild
			1 week	80°C	88 (12)	Mild
			3 months	80°C	84 (16)	Moderate
5	Photolytic (sunlight)	7 µg/mL of raltegravir	3 h	25°C, 60 RH	94 (6)	Mild
			1 week	25°C, 60 RH	91 (9)	Mild
			3 months	40°C, 75 RH	88 (12)	Moderate

Table 8: System suitability and validation parameters of raltegravir

Serial number	Parameter	Result
1	USP plate count	9,677
2	USP tailing	1.626
3	% RSD	0.011546
4	Accuracy	98–100%
5	Precision (%RSD)	
	Method precision	
	Ruggedness	<2%
6	Linearity	3.0–15 µg/mL
7	Correlation coefficient	0.999
8	LOD	4.8 µg/mL
9	LOQ	1.6 µg/mL
10	Robustness (tailing factor)	
	Flow rate –	1.54
	Flow rate +	1.56
	More organic phase	1.54
	Less organic phase	1.53

LOQ: Quantification, LOD: Limits of detection, RSD: Relative standard deviation

with mean recovery values ranging from 100.01% to 100.67%, which are well within the acceptable limits. The method also exhibited excellent sensitivity, with a LOD of 1.6 µg/mL and a LOQ of 4.8 µg/mL, ensuring reliable detection of raltegravir at low concentrations. Robustness studies were performed by varying chromatographic conditions, such as flow rate and mobile phase composition. The results demonstrated the method's stability under minor changes, as indicated by retention time consistency, acceptable tailing factor values, and theoretical plate counts within regulatory standards. Forced degradation studies confirmed that the method could effectively differentiate between the drug and its degradation products, with degradation levels ranging from 5% to 20%, in accordance with ICH Q1B guidelines.

The forced degradation study of raltegravir shows that in acidic conditions (0.1 N HCl), the drug retains 95% potency after 3 h, with only slight degradation observed over time, indicating no significant interference with degradants. In contrast, under alkaline conditions (NaOH, 0.1 N) and thermal conditions, the stability declined more rapidly, dropping to 92% and 91%, reflecting significant degradation. Similarly, photolytic and peroxide exposure revealed gradual degradation, with the assay content at 94% after 3 h, again showing no significant interference with degradants according to the ICH guidelines (Q1A, Q1B, and Q2B).

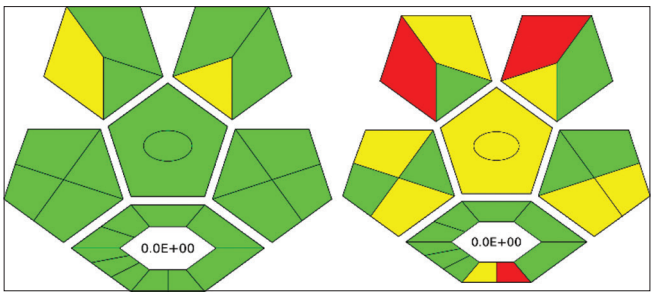

Fig. 5: Raltegravir with acetonitrile and ethanol green metrics using complex green analytical procedure index

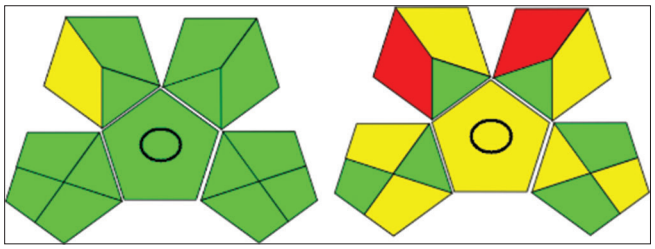

Fig. 6: Raltegravir with acetonitrile and ethanol green metrics using green analytical procedure index

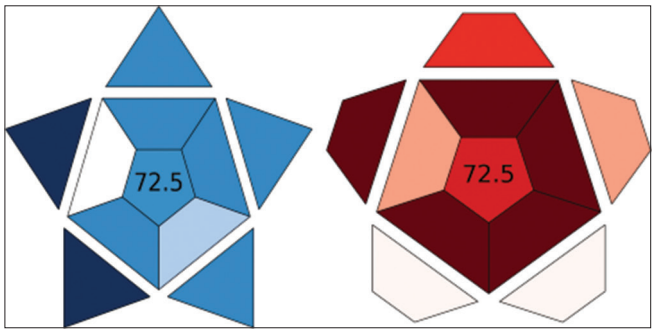

Fig. 7: Raltegravir with blue applicability grade index and red analytical performance index indicating practicability and efficiency

Significance of ethanol and green metric tools

The proposed method utilizes the green solvent ethanol, which is listed as eco-friendly by the U.S.Tri-E.P.A [46]. The sample handling,

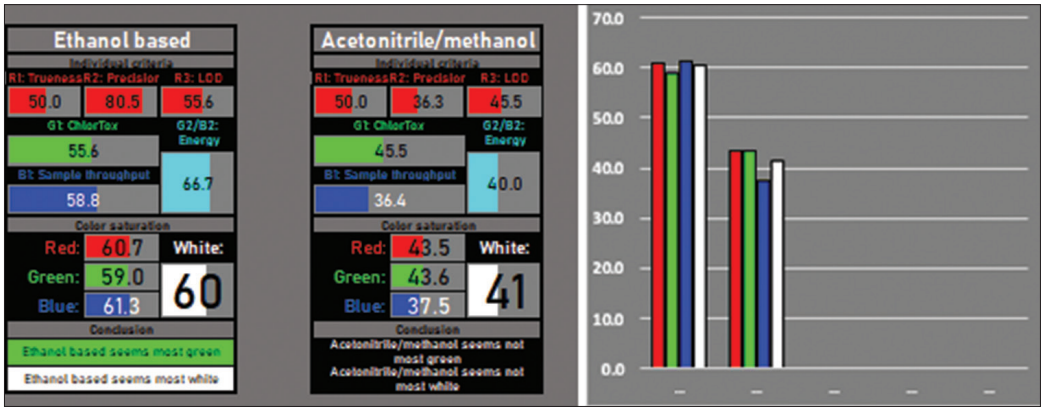

Fig. 8: Comparison of conventional methods with ethanol-based method

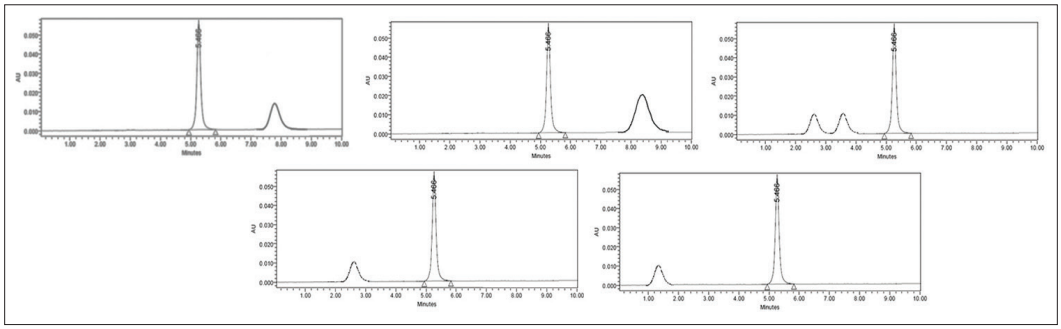

Fig. 9: Stress testing studies of raltegravir

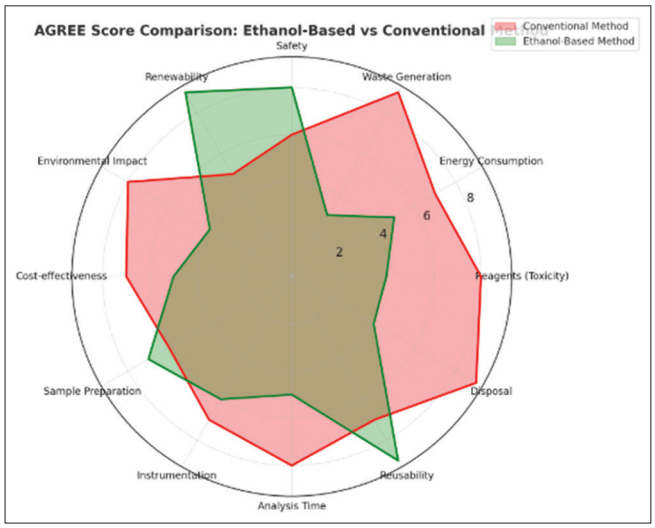

Fig. 10: Comparison of conventional method with ethanol-based method of analytical greenness metric approach

waste management, and utilization of other resources comply with the 12 green analytical principles. "AGREE, GAPI, Complex GAPI, BAGI, RAPI, and RGB" demonstrated exceptional results of score near 1 and green hexagonal charts indicating that ethanol is more reliable for analysis of our drug. Similar evaluations have been reported in the literature for remdesivir analysis, where green metrics were compared using AGREE, GAPI, and other tools [47] and similar estimations have been reported for ritonavir and ombitasvir by UPLC using ethanol as a green solvent [48]. The quantitative analysis of favipiravir and remdesivir employed ethanol, a green solvent, for spectrophotometric analysis, aligned with green chemistry principles [49]. The FT-IR-based method for entecavir quantification adopts green chemistry principles by avoiding the use of toxic reagents and environmentally friendly procedures. This approach demonstrates high precision, accuracy, and linearity, making it a sustainable alternative for pharmaceutical analysis [50]. This reinforces the importance of adopting ethanol-based methods owing to their proven greenness and sustainability.

Comparison of reported methods with proposed method

Several analytical methods have been reported for the quantification of raltegravir, using HPLC with methanol/acetonitrile as the organic solvent. While effective, these conventional approaches pose significant environmental and health hazards owing to toxicity, non-renewability, and disposal challenges. The proposed method also maintains a high analytical performance compared to existing methods. Thus, this work presents a viable greener alternative for the regular analysis of raltegravir aligning with environmentally responsible analytical methodologies. By contrast, the proposed method introduces a greener alternative by employing ethanol as an organic modifier, thereby reducing the overall environmental footprint. The shift was visualized using Matplotlib, as shown in the AGREE score chart in Fig. 10. Highlighting the ethanol-based method's reduced environmental impact and toxicity, as well as the GAPI and Complex GAPI score charts, further reinforcing its superior performance across various sustainability criteria, as shown in Fig. 11. The adoption of ethanol instead of conventional solvents is a safer, more sustainable, and cost-effective alternative. Ethanol is derived from renewable sources and exhibits lower toxicity making it an ideal greener solvent and triethylamine eliminates phosphate-related concerns in the environment such as eutrophication in water systems [51].

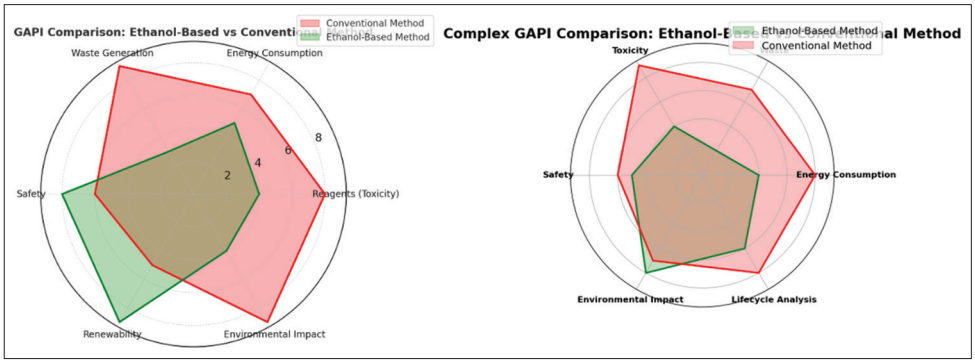

Fig. 11: Comparison of conventional method with ethanol-based method of green analytical procedure index and complex green analytical procedure index

CONCLUSION

A novel, simple, precise, and stable green solvent-assisted analytical method was developed to quantify raltegravir in "bulk and pharmaceutical dosage forms." The method aligns with ICH guidelines demonstrating the linear regression with an r^2 value of 0.999 in the range of 3.0 µg/mL–15 µg/mL. The method's accuracy and precision are validated by percentage RSD values under 2 %, with a % recovery range of 98–100%. The LOD and LOQ were within acceptable standards. Stress testing studies show that the % degradation of the active ingredient remains between 5 and 20%, meeting the pre-defined acceptance criteria.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

ACKNOWLEDGMENT

The authors are thankful to the management of the Vels Institute of Science, Technology, and Advanced Studies (VISTAS), Pallavaram, Chennai-600 117, Tamil Nadu, India, for providing the research facility.

AUTHORS' CONTRIBUTIONS

Dr. M. Sumithra – Corresponding author, methodology, design, data analysis, review of original draft. K. Archana – First author, Implementation of project, writing original draft.

COMPETING INTERESTS

There are no competing interests.

FUNDING

No funding was acquired or granted.

REFERENCES

1. Kaya SI, Cetinkaya A, Ozkan SA. Green analytical chemistry approaches on environmental analysis. Trends Environ Anal Chem. 2022;33:e00157.
2. Sajid M, Płotka-Wasylka J. Green analytical chemistry metrics: A review. Talanta. 2022;238:123046.
3. López-Lorente ÁI, Pena-Pereira F, Pedersen-Bjergaard S, Zuin VG, Ozkan SA, Psillakis E. The ten principles of green sample preparation. TrAC Trends Anal Chem. 2022;148:116530.
4. Winterton N. The green solvent: A critical perspective. Clean Technol Environ Policy. 2021;23(9):2499-522.
5. Prasad W, Wani AD, Khamrui K, Hussain SA, Khetra Y. Green solvents, potential alternatives for petroleum based products in food processing industries. Cleaner Chem Eng. 2022;3:100052.
6. Usman M, Cheng S, Boonyubol S, Cross JS. Evaluating green solvents for bio-oil extraction: Advancements, challenges, and future perspectives. Energies. 2023;16(15):5852.
7. Kim HJ, Kim YJ, Han GS, Jung HS. Green solvent strategies toward sustainable perovskite solar cell fabrication. Sol RRL. 2024;8(5):2300910.
8. Chentouf A. Toxicological evaluation of complex mixtures: Prediction and interactions-A review. J Pharm Pharmacol Res. 2022;5(3):17-20.
9. Dorman FL, Reiner EJ. Gas chromatographic analysis of emerging and persistent environmental contaminants. In: Gas Chromatography. Netherlands: Elsevier; 2021. p. 835-64.
10. Organisation for Economic Co-operation and Development (OECD). SIDS Initial Assessment Report for SIAM 19. Ethanol. Kenya: UNEP Publishers; 2004.
11. Carraschi SP, Cruz C, Shiogiri NS, Pitelli RA, Silva AF. Sensitivity of aquatic organisms to ethanol and its potential use as bioindicators. Acta Sci Biol Sci. 2018;40(1):e39392.
12. Ma B, Yan Y, Wu M, Li S, Ru M, Xu Z, *et al*. Sustainable solution processing toward high-efficiency organic solar cells: A comprehensive review of materials, strategies, and applications. Adv Funct Mater. 2025;35(3):2413814.
13. Inert Reassessment - Three Exemptions from the Requirement of a Tolerance for Ethyl Alcohol (CAS #64-17-5). United States: Environmental Protection Agency (EPA);2024.
14. McElvain SM, Fanta PD. Ethanol and green chemistry: Applications and environmental benefits. J Clean Prod. 2004;12(8):1021-30.
15. Tundo P, Stabile R. Green solvents for organic synthesis: The role of ethanol. Chem Soc Rev. 2010;39(9):3653-65. doi: 10.1039/B915546K
16. Fang Y, Zhang L, Sun L. Ethanol as a green solvent: Characteristics and applications. Environ Sci Technol. 2015;49(16):9953-61.
17. Chemat F, Abert-Vian M, Fabiano-Tixier AS. Green Solvents and Sustainable Processes for Green Chemistry. London: IntechOpen; 2012.
18. Gamal M, Naguib IA, Panda DS, Abdallah FF. Comparative study of four greenness assessment tools for selection of greenest analytical method for assay of hyoscine n-butyl bromide. Anal Methods. 2021;13:369-80.
19. Mohamed HM. Green, environment-friendly, analytical tools give insights in pharmaceutical and cosmetics analysis. TrAC Trends Anal Chem. 2015;66:176-92.
20. Mansour FR, Omer KM, Płotka-Wasylka J. A total scoring system and software for complex modified GAPI (ComplexMoGAPI) application in the assessment of method greenness. Green Anal Chem. 2024;10:100126.
21. Płotka-Wasylka J, Wojnowski W. Complementary green analytical procedure index (ComplexGAPI) and software. Green Chem. 2021;23(21):8657-65.
22. Macioszek VK, Sobczak M, Skoczowski A, Oliwa J, Michlewska S, Gapinska M, *et al.* The effect of photoperiod on necrosis development,

photosynthetic efficiency and 'green islands' formation in *Brassica juncea* infected with *Alternaria brassicicola*. Int J Mol Sci. 2021;22:8435.
23. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White analytical chemistry: An approach to reconcile the principles of green analytical chemistry and functionality. TrAC Trends Anal Chem. 2021;138:116223.
24. Patel S, Nagappan K, Reddy GS, Kowmudi G. Quantitative reverse-phase high-performance liquid chromatographic method for the quantification of raltegravir potassium in bulk and dosage forms. J Young Pharm. 2019;11(3):274-8.
25. Shirisha V, Sairaju B, Illendula S, Dutt KR. Development and validation of stability indicating RP-HPLC assay method of raltegravir in tablet dosage forms. World J Pharm Pharm Sci. 2019;8:668-92.
26. Coskun O. Separation techniques: Chromatography. North Clin Istanb. 2016;3(2):156-60.
27. 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.
28. Tejaswi JK, 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 Appl Pharm. 2018;10(6):242-8.
29. Atmakuri LR, Raghu Ram MS. Validated reverse phase HPLC method for determination of raltegravir in pharmaceutical preparations. Int J Res Pharm Chem. 2012;2(1):217-21.
30. Patel PA, Patel ZN, Patel MM. Development and validation of RP-HPLC method for estimation of raltegravir potassium in bulk and pharmaceutical dosage form. J Chem Pharm Res. 2013;5(5):185-90.
31. Nandhakumar L, Parasuraman S, Ilavarasan R. Development and validation of RP-HPLC method for determination of raltegravir in bulk and pharmaceutical dosage form. Int J Drug Dev Res. 2013;5(3):297-302.
32. Nageswara Rao R, Narasa Raju A, Anjaneyulu Y. A new stability-indicating RP-HPLC method for the determination of raltegravir in bulk and its pharmaceutical dosage forms. J Chromatogr Sci. 2014;52(1):27-34.
33. Teevan Y, Naik M, Kotapalli S, Nadendla R. Development and validation of a stability-indicating RP-HPLC method for the quantitative estimation of raltegravir in bulk and pharmaceutical dosage forms. J Pharm Res. 2016;10(1):52-8.
34. Nanda RK, Banerjee SK. Development and validation of stability-indicating RP-HPLC method for determination of raltegravir in tablet dosage form. Int J PharmTech Res. 2013;5(2):630-7.
35. Benzigar S, Divya T, Sujith V, Manavalan K. Development and validation of RP-HPLC method for the estimation of raltegravir potassium in bulk and dosage forms. Res J Pharm Technol. 2010;3(4):1244-7.
36. Jabbaribar F, Mortazavi A, Jalali-Milani R, Jouyban A. Analysis of Oseltamivir in Tamiflu capsules using micellar electrokinetic chromatography. Chem Pharm Bull (Tokyo). 2008;56(12):1639-44.
37. Voges AB, Martina ZJ, Alberto FC. A green analytical chemistry method for the determination of oseltamivir in capsules by capillary electrophoresis with micellar electrokinetic chromatography. Microchem J. 2017;132:102-8.
38. Stuart JD, Pungpat J, Suppasansatorn P. Green RP-HPLC for the analysis of oseltamivir in pharmaceutical formulations. Anal Methods. 2014;6(11):3726-31.
39. Basavaiah K, Swamy CN, Kumar UV. Green RP-HPLC method for the simultaneous estimation of Acyclovir and its impurities in pharmaceutical formulation. J Chromatogr Sci. 2015;53(4):573-9.
40. Ferreira MM, Brzezinska E, Sobanska AW, Pyzowski J. Development of an eco-friendly RP-HPLC method for the estimation of zidovudine using green solvents. Curr Pharm Anal. 2018;14(3):203-8.
41. Roy S, Siddique S, Majumder S, Abdul MI, Rahman SA, Lateef D, *et al*. A systemic approach on understanding the role of moisture in pharmaceutical product degradation and its prevention: Challenges and perspectives. Biomed Res. 2018;29:3336-43.
42. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. J Appl Pharm Sci. 2012;2:129-38.
43. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, Parashar N, *et al*. Forced degradation studies to assess the stability of drugs and products. TrAC Trends Anal Chem. 2013;49:71-88.
44. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik T, Motto MG. Conducting forced degradation studies. Pharm Technol. 2002;26(2):48-56.
45. Bassi P, Kaur G. pH modulation: A mechanism to obtain pH-independent drug release. Expert Opin Drug Deliv. 2010;7(7):845-57.
46. Ullah N, Haseeb A, Tuzen M. Application of recently used green solvents in sample preparation techniques: A comprehensive review of existing trends, challenges, and future opportunities. Crit Rev Anal Chem. 2023;54(8):2714-33. doi: 10.1080/10408347.2023.2197495
47. Bhalodia A, Desai S. Application of seven tools for evaluation of greenness of chromatographic methods for remdesivir analysis. Green Anal Chem. 2024;11:100149.
48. Kumar BP, Pasha TY. Green assessment of analytical procedure for the determination of anti-retro viral drugs by HPLC. Int J Pharm Invest. 2024;14(4):1147-54.
49. Batubara AS, Abdelazim AH, Almrasy AA, Gamal M, Ramzy S. Quantitative analysis of two COVID-19 antiviral agents, favipiravir and remdesivir, in spiked human plasma using spectrophotometric methods; Greenness evaluation. BMC Chem. 2023;17:58.
50. Anusha M, Gubbiyappa KS. Bioanalytical method development and validation for the determination of Asciminib anticancer drug in biological matrices by LC–ESI-MS/MS. Int J Appl Pharm. 2024;16(6):176-81
51. Bhutia GT, Kumar De A, Bera T. Validation, stability studies, and simultaneous estimation of co-encapsulated curcumin, epigallocatechin gallate nanoformulation by RP-HPLC method. Int J Appl Pharm. 2022;14:186-95.