The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1): 819-828, 2026 www.thebioscan.com

# STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ANTIRETROVIRAL DRUGS IN BULK AND TABLET FORMULATIONS

**SUBHRANSHU PANDA, SIRISHA GORANTLA***

Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Jaipur National University, Jagatpura, Jaipur, Rajasthan 302017, India

**Corresponding Author:** Sirisha Gorantla, **Email:** sirishagorantla24@gmail.com

**DOI: 10.63001/tbs.2026.v21.i01.pp819-828**

**Keywords**
Abacavir, Dolutegravir, Lamivudine, RP-HPLC, method validation, stability-indicating, forced degradation, ICH Q2(R1)

**Received on:**

**30-11-2025**

**Accepted on:**

**29-12-2025**

**Published on:**

**01-02-2026**

**ABSTRACT**

A reliable, validated, and robust RP-HPLC method was established to evaluate Abacavir (ABV), Dolutegravir (DTV), and Lamivudine (LVD) in bulk and fixed-dose combination tablets. Chromatographic separation was achieved on a Sunfire C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of 0.01 N ammonium acetate:acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min, with detection at 257 nm. The column temperature was maintained at 30°C and the run time was 6 min. The method was linear over 0-180 µg/mL for ABV, 0-15 µg/mL for DTV, and 0-90 µg/mL for LVD with correlation coefficients ($R^2$) of 0.9993, 0.9998, and 0.9998, respectively. Accuracy was within 98.15-101.35% and precision showed %RSD < 1%. The LOD/LOQ values were 0.746/2.261 µg/mL for ABV, 0.057/0.172 µg/mL for DTV, and 0.293/0.888 µg/mL for LVD. Forced degradation showed maximum degradation of 6.83% (ABV), 8.01% (DTV), and 5.28% (LVD), confirming specificity and suitability for stability and routine QC.

## INTRODUCTION

HIV can be effectively managed through the application of combination therapy[1]. The activity of HIV-1 reverse transcriptase[2-3] is proficiently inhibited by abacavir, which possesses the chemical formula $C_{14}H_{18}N_6O$. Dolutegravir[4], an FDA-approved integrase strand transfer inhibitor, is employed in the treatment of HIV infection. The viral integrase enzyme executes a two-step process that specifically obstructs the strand transfer phase of the viral genome's integration into the host cell's DNA. Its chemical formula is $C_{20}H_{19}F_2N_3O_5$, and it demonstrates solubility in both water and methanol. Lamivudine exhibits enhanced potency and targets both the Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) by inhibiting the reverse transcriptase enzyme. The molecular formula for lamivudine is

$C_8H_{11}N_3O_3S$, and it showcases excellent solubility in water, low solubility in methanol, and negligible solubility in acetone. According to the literature, abacavir (ABV), dolutegravir (DTV), and lamivudine (LVD) can be measured both individually and concurrently in bulk and mixed-dose forms utilizing chromatographic techniques and HPLC-MS/MS[5-8]. The work reveals that the specificity, sensitivity, accuracy, and precision of the RP-HPLC technique are commendably stable. The developed solution adhered to ICH Q2 R1 criteria, which were successfully validated. There have been limited reports of simultaneous analytical techniques employing HPLC on these combination dosage forms; following the application of specific stress conditions, the current technique demonstrated remarkable linearity, precision, accuracy, and stability.

## Materials and Methods

### Instruments Required

A 3098 Photo Diode Array (PDA) detector, Empower 3 software were utilized to execute an RP-HPLC technique on a Waters Alliance 2695 HPLC system. A Sunfire C18 stationary phase measuring 4.6 × 250 mm and 5 µm was utilized. A Denver electronic balance, Whatman filter paper No. 41, and an ultrasonic bath sonicator called a Frontline FS 4, were utilized in this work.

### Reagents Used

Hetero Drugs Limited in Hyderabad, India, supplied lamivudine, dolutegravir, and abacavir. HPLC-grade acetonitrile and ammonium acetate were procured from standard laboratory suppliers, and Milli-Q water was used.

### Reagents and Chemicals

Analytical and HPLC-grade chemicals were used. Water underwent double distillation and membrane filtration. The mobile phase was prepared using 0.01 N ammonium acetate (aqueous buffer) and HPLC-grade acetonitrile in the ratio 60:40 v/v. The tablets (ABV 600 mg, LVD 300 mg, and DTV 50 mg) were procured locally.

### Preparation of Standard Solution

10 mg each of Abacavir (ABV), Dolutegravir (DTV), and Lamivudine (LVD) were dissolved in the diluent (water:acetonitrile, 50:50 v/v) and diluted to a final volume of 10 mL to achieve stock solutions of 1000 µg/mL. A working solution was made by combining 1.5 mL of ABV, 0.125 mL of DTV, and 0.75 mL of LVD stock solutions in a 10 mL flask, followed by dilution to volume with the mobile phase, resulting in concentrations of ABV: 150 µg/mL,

DTV: 12.5 µg/mL, and LVD: 75 µg/mL. The solution was maintained at a temperature between 2 and 8 °C in amber vials[9-11.]

**Selection of Mobile Phase**

The optimal mobile phase for separating Abacavir, Dolutegravir, and Lamivudine was found to be 0.01 N ammonium acetate:acetonitrile (60:40 v/v), providing sharp, well-resolved peaks with high theoretical plates, minimal tailing, and good peak areas.

**Sample Preparation**

An aliquot equivalent to twenty tablets was gently crushed and added to a 10 mL volumetric flask. Using the diluent (water:acetonitrile, 50:50 v/v), the resultant mixture was sonicated for 5 minutes before being filtered through a 0.45 µm membrane. By diluting the filtrate accordingly, the final concentrations for LVD, DTV, and ABV were 300, 25, and 150 µg/mL, respectively. For analysis, a 10 µL sample was injected into the HPLC system[12-14]. The sample peak areas were compared with those of standard solutions at identical concentrations.

## RESULTS AND DISCUSSION

**Chromatographic Conditions**

Using a mobile phase consisting of 0.01 N ammonium acetate and acetonitrile at a ratio of 60:40 (v/v), chromatographic separation was carried out on a Sunfire C18 column (4.6 × 250 mm, 5 µm) maintained at 30°C. The flow rate of the mobile phase was set at 1.0 mL/min, the detection wavelength was 257 nm, the injection volume was 10 µL, and the run time was 6 min. Prior to utilization, the mobile phase was degassed and filtered through a 0.45 µm membrane [15-18]. Standard solutions were accurately aliquoted into Amber vials for subsequent analysis.

**Method Validation**

The International Council for Harmonization's (ICH) recommended HPLC method was verified against Q2 (R1) criteria for analytical method validation [19-20].

**System Suitability Study**

The system suitability investigation, Table 1 displays the good peak areas and theoretical plates of >5000 and a good tailing factor of <2. In Figure 1, the chromatograms are shown.

**Table-1: System Suitability Parameter for Abacavir, Dolutegravir, and Lamivudine**

| Parameter | Abacavir | Dolutegravir | Lamivudine |
|---|---|---|---|
| Retention Time (Min.) | 2.199 | 2.648 | 3.195 |

| | | | |
|---|---|---|---|
| Peak Area (%RSD) | 0.6 | 0.7 | 1.3 |
| Tailing Factor | 1.08 | 1.11 | 1.10 |
| Theoretical Plates | 5450 | 6125 | 5890 |
| Resolution | -- | 4.32(vs Abacavir) | 3.25(vs Dolutegravir) |

%RSD: Percentage Relative standard deviation

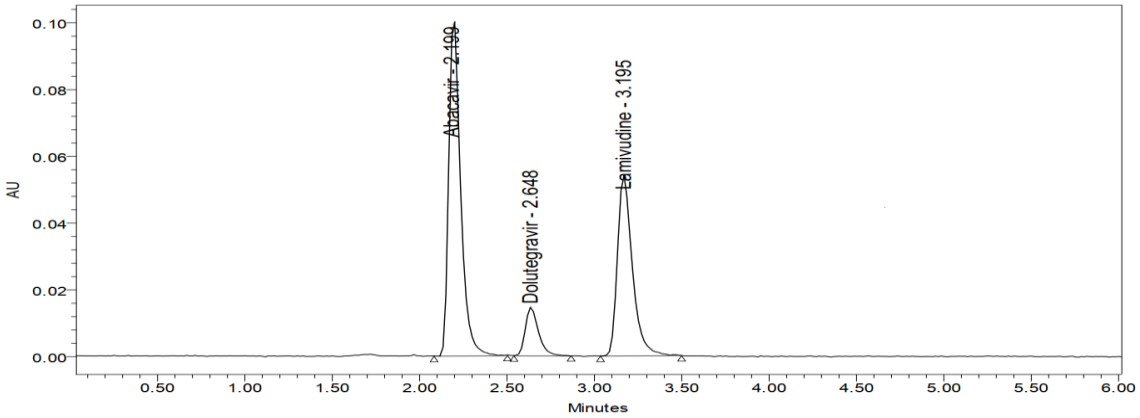


**Fig.-1: Standard Chromatogram**

**Linearity**

Standard solutions were prepared to obtain linear concentrations of ABV, DTV, and LVD in the ranges of 0-180 µg/mL, 0-15 µg/mL, and 0-90 µg/mL, respectively (Figure 2). The calibration plots of concentration versus peak area showed good linearity across the studied ranges with correlation coefficients ($R^2$) of 0.9993 (ABV), 0.9998 (DTV), and 0.9998 (LVD) (Table 2).

**Table-2: Results of Linearity Studies**

| S. No | Abacavir µg/mL | Peak Area | Dolutegravir µg/mL | Peak Area | Lamivudine µg/mL | Peak Area |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 30 | 155570 | 2.5 | 23160 | 15 | 83171 |
| 3 | 60 | 325531 | 5 | 47080 | 30 | 166448 |
| 4 | 90 | 453209 | 7.5 | 69618 | 45 | 242030 |
| 5 | 120 | 606245 | 10 | 93418 | 60 | 328714 |
| 6 | 150 | 755329 | 12.5 | 116599 | 75 | 403560 |
| 7 | 180 | 906405 | 15 | 137966 | 90 | 484231 |

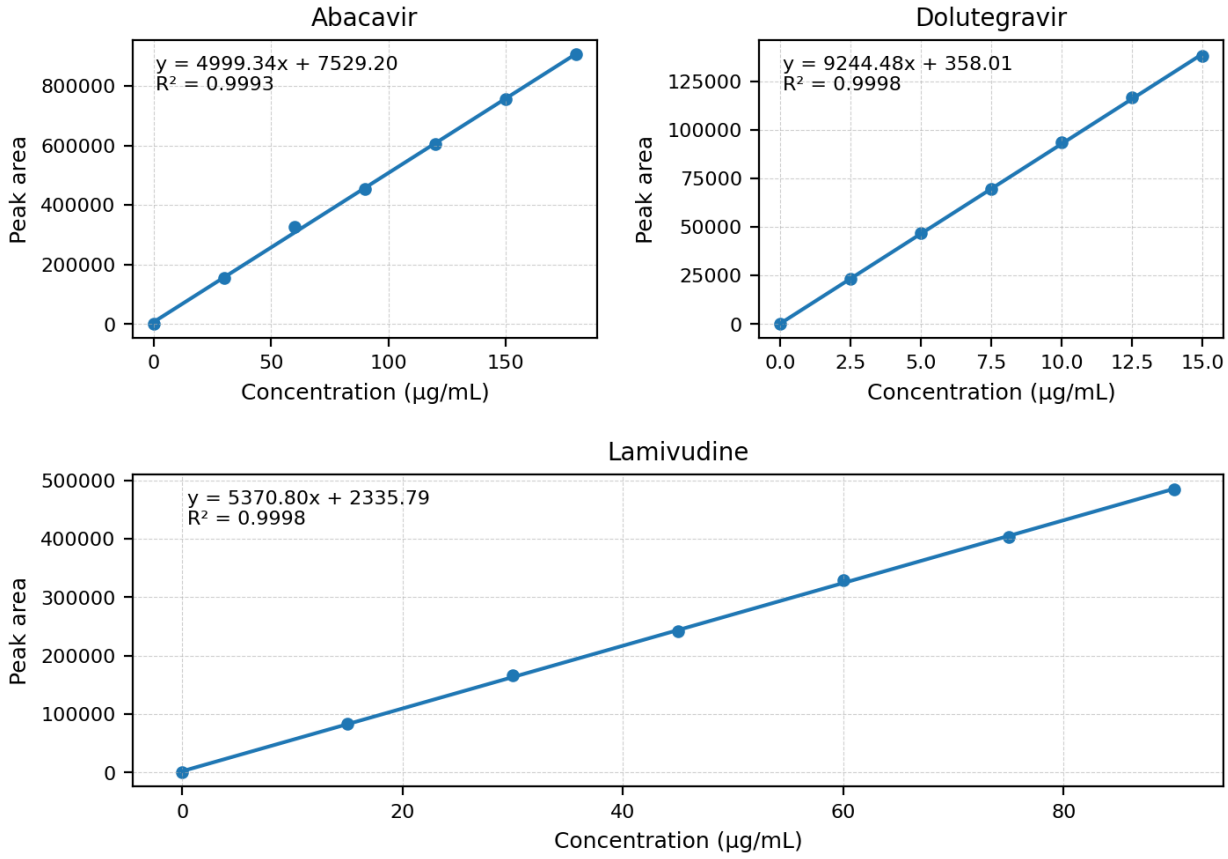


**Fig.-2: Calibration Graph of Abacavir, Dolutegravir, and Lamivudine**

**Precision**

Evaluations were conducted utilizing system precision, repeatability, and intermediate precision to assess the proposed HPLC method's precision in accordance with ICH Q2(R1). System precision showed %RSD values of 0.447% (ABV), 0.748% (DTV), and 0.611% (LVD) (Table 3). Repeatability gave %RSD values of 0.313% (ABV), 0.276% (DTV), and 0.649% (LVD) (Table 4). Intermediate precision (different day) showed %RSD values of 0.352% (ABV), 0.204% (DTV), and 0.953% (LVD) (Table 5).

**System Accuracy**

**Table-3: System precision of Abacavir, Dolutegravir, and Lamivudine**

| Concentration (µg/mL) | **Abacavir** | **Dolutegravir** | **Lamivudine** |
|---|---|---|---|
| | 60 | 5 | 30 |
| Area (Mean±SD) | 591775.33±2647.55 | 92093.50±689.17 | 316491.33±1932.74 |
| %RSD | 0.447 | 0.748 | 0.611 |

All values are expressed as Mean±SD, n=6 %RSD: Percentage Relative standard deviation

**Table-4: Repeatability table of Abacavir, Dolutegravir, and Lamivudine**

| Concentration (µg/mL) | **Abacavir** | **Dolutegravir** | **Lamivudine** |
|---|---|---|---|
| | 60 | 5 | 30 |
| Area (Mean±SD) | 591369.00±1850.19 | 92164.33±254.08 | 314959.00±2045.50 |
| %RSD | 0.313 | 0.276 | 0.649 |

All values are expressed as Mean±SD, n=6 %RSD: Percentage Relative standard deviation

**Table-5: Abacavir, Dolutegravir, and Lamivudine Intermediate precision**

| Concentration (µg/mL) | **Abacavir** | **Dolutegravir** | **Lamivudine** |
|---|---|---|---|
| | 60 | 5 | 30 |
| Area (Mean±SD) | 592106.33±2085.00 | 89165.00±182.17 | 309474.17±2949.94 |
| %RSD | 0.352 | 0.204 | 0.953 |

All values are expressed as Mean±SD, n=6 %RSD: Percentage Relative Standard Deviation

**Accuracy**

The method's accuracy was evaluated by recovery studies at 50%, 100%, and 150% levels. Recoveries ranged from 99.26 to 99.96% for ABV, 98.15 to 100.67% for DTV, and 99.20 to 101.35% for LVD, confirming acceptable accuracy (Table 6).

**Table-6: Accuracy Results**

| **Drug** | **Level %** | **Spiked** | **Recovered** | **% Recovered** |
|---|---|---|---|---|
| Abacavir | 50% | 60 | 59.82 | 99.70 |
| | 100% | 120 | 119.51 | 99.59 |
| | 150% | 180 | 179.26 | 99.59 |
| Dolutegravir | 50% | 5 | 4.94 | 98.89 |
| | 100% | 10 | 9.95 | 99.45 |
| | 150% | 15 | 15.03 | 100.22 |
| Lamivudine | 50% | 30 | 30.01 | 100.03 |
| | 100% | 60 | 60.09 | 100.15 |
| | 150% | 90 | 89.68 | 99.64 |

**Robustness**

The robustness of the methodology was assessed by manipulating the wavelength (±2 nm) and flow rate (±0.2 mL/min). The %RSD values consistently remained below 2% (0.12–0.28% for wavelength, 0.06–0.3% for flow rate), thereby affirming the method's dependability amidst minor intentional variations, as illustrated in Table 7.

**Table-7: Robustness Data**

| Condition | Drug | Mean Area | S.D | %RSD | Acceptance Criteria |
|---|---|---|---|---|---|
| Wavelength 255 nm | Abacavir | 871140.0 | 1236.85 | 0.14% | < 2% |
| | Dolutegravir | 312478.33 | 635.27 | 0.2% | |
| | Lamivudine | 202635.33 | 471.74 | 0.23% | |
| Wavelength 259 nm | Abacavir | 871304.67 | 1027.97 | 0.12% | |
| | Dolutegravir | 312862.67 | 841.63 | 0.27% | |
| | Lamivudine | 202751.0 | 575.11 | 0.28% | |
| Flow rate 0.8 mL/min | Abacavir | 870514.0 | 690.27 | 0.08% | |
| | Dolutegravir | 312285.33 | 949.77 | 0.3% | |
| | Lamivudine | 202664.0 | 414.09 | 0.2% | |
| Flow rate 1.2 mL/min | Abacavir | 871377.67 | 664.57 | 0.08% | |
| | Dolutegravir | 312111.0 | 326.22 | 0.1% | |
| | Lamivudine | 202764.0 | 123.12 | 0.06% | |

SD: Standard deviation, %RSD: Percentage Relative standard deviation

**Limits of Quantification (LOQ) and Detection (LOD)**

Using the calibration curve slope (S) and standard deviation (σ), LOD and LOQ were calculated as per ICH Q2(R1). Dolutegravir showed the highest sensitivity with the lowest LOD/LOQ (0.057 µg/mL and 0.172 µg/mL), followed by lamivudine (0.293 µg/mL and 0.888 µg/mL). Abacavir showed LOD/LOQ of 0.746 µg/mL and 2.261 µg/mL. These results confirm that the method is sufficiently sensitive for routine analysis (Table 8).

**Table-8. LOD and LOQ**

| Drug | Slope (S) | Standard Deviation (σ) | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|
| Abacavir | 4999.33 | 1130.41 | 0.746 | 2.261 |
| Dolutegravir | 9244.50 | 158.69 | 0.057 | 0.172 |
| Lamivudine | 5366.07 | 476.28 | 0.293 | 0.888 |

**Assay**

Table 9 displays the assay findings for the active substances, which were determined to be within acceptable bounds.

**Table-9: Assay Results**

| Drug | Label Claim (mg) | Assay (%) |
|---|---|---|
| Abacavir | 600 | 99.73 |
| Dolutegravir | 50 | 99.88 |
| Lamivudine | 300 | 99.42 |

**Force Degradation**

Forced degradation studies were performed under acidic (0.1 N HCl), basic (0.1 N NaOH), oxidative (3% H2O2), thermal, photolytic (UV), and neutral (water) conditions to assess specificity and stability-indicating capability. The observed assay values were 93.17-99.35% for ABV, 91.99-99.66% for DTV, and 94.72-99.09% for LVD, corresponding to maximum degradations of 6.83%, 8.01%, and 5.28%, respectively (Table 10). These results demonstrate that the method can separate analyte peaks from degradation products.

**Table-10: Forced Degradation Results**

| Stress Condition | ABV % Assay | ABV % Degradation | DTV % Assay | DTV % Degradation | LVD % Assay | LVD % Degradation |
|---|---|---|---|---|---|---|
| Acid (0.1N HCl) | 93.17 | 6.83 | 92.34 | 7.66 | 94.91 | 5.09 |
| Base (0.1N NaOH) | 96.48 | 3.52 | 94.47 | 5.53 | 96.00 | 4.00 |
| Oxidative (H2O2) | 95.23 | 4.77 | 91.99 | 8.01 | 94.72 | 5.28 |
| Thermal | 98.32 | 1.68 | 97.22 | 2.78 | 97.34 | 2.66 |
| Photolytic (UV) | 98.06 | 1.94 | 98.41 | 1.59 | 98.08 | 1.92 |
| Neutral (Water) | 99.35 | 0.65 | 99.66 | 0.34 | 99.09 | 0.91 |

The degradation results indicate that all three compounds experienced measurable degradation under stress conditions, with the most significant degradation observed under

basic and acidic hydrolysis. Dolutegravir showed relatively better stability in acidic and oxidative conditions. The observed degradation levels were within acceptable limits (below 15%), verifying that the HPLC technique developed is appropriate for use as a stability-indicating method.

## CONCLUSION

In compliance with ICH Q2(R1) guidelines, an RP-HPLC method was developed and validated for simultaneous estimation of Lamivudine (LVD), Dolutegravir (DTV), and Abacavir (ABV) in tablets. The method showed excellent linearity ($R^2 \geq 0.9993$) over 0-90 µg/mL (LVD), 0-15 µg/mL (DTV), and 0-180 µg/mL (ABV), with good accuracy (98.15-101.35%), precision (%RSD < 1%), and low LOD/LOQ values (Table 8). Forced degradation results confirmed the method as stability-indicating, and the proposed method is suitable for routine quality control and stability studies.

## CONFLICTS OF INTEREST

Not applicable

## FUNDING

This research didn't receive any funding from any agencies.

## AUTHORS CONTRIBUTIONS

Sirisha Gorantla: planning, conceptualization, data collection and paper writing. Subhranshu Panda: review of literature and data interpretation. The research profile of the authors can be verified from their ORCID ids, given below:

Subhranshu Panda: https://orcid.org/0000-0003-4782-8895

Sirisha Gorantla: https://orcid.org/0009-0000-4360-438X

## REFERENCES

1. P. Cahn, J. S.Madero, J. R. Arribas, A. Antinori, R. Ortiz, A. E. Clarke *et al.*, *Lancet*, **393(10167),** 143-155(2019), https://doi.org/10.1016/S0140-6736(18)32462-0.
2. B. A. Santevecchi, S. Miller, L. M. Childs-Kean, *Ann Pharmacother*, **54(12),** 1252-1259(2020), https://doi.org/10.1177/1060028020933772.
3. M. Doffito, L. Waters, P. Cahn, R. Paredes, J. Koteff, J. Van Wyk, *AIDS Res Hum Retroviruses*, **36(1),** 13-18(2020), https://doi.org/10.1089/AID.2019.0171.
4. K. Joshi, S. Shinde, S. Karatela, A. Mulkalwar, *Cureus*, **16(6),** e62522(2024), https://doi.org/10.7759/cureus.62522.

5. P. Cahn, A. L. Pozniak, H. Mingrone, A. Shuldyakov, C. Brites, J. F. Andrade-Villanueva, *et al.*, *Lancet HIV*, **2(4),** e127–e136(2015). https://doi.org/10.3389/fimmu.2022.1033098.
6. S. Min, L. Sloan, E. DeJesus, T. Hawkins, L. McCurdy, I. Song, et al., *AIDS*, **25(14),** 1737–1745(2011). https://doi.org/10.1097/QAD.0b013e32834a1dd9.
7. F. Raffi, H. Jaeger, E. Quiros-Roldan, H. Albrecht, E. Belonosova, J. M. Gatell, *Lancet Infect Dis*, **13(11),** 927-35(2013), https://doi.org/10.1016/S1473-3099(13)70257-3.
8. S. L. Walmsley, A. Antela, N. Clumeck, D. Duiculescu, A. Eberhard, F. Gutiérrez, *N Engl J Med*, **369(19),** 1807-18(2013), https://doi.org/10.1056/NEJMoa1215541.
9. S. Swarnalatha, N. Vallakeerthi, R. S. Moorthy, K. Pingili, P. M. Reddy, *Asian J Green Chem*, **8(4),** 478-496(2024), https://doi.org/10.48309/AJGC.2024.454781.1497.
10. R. Zash, L. Holmes, M. Diseko, D. L. Jacobson, S. Brummel, G. Mayondi, *N Engl J Med*. **381(9),** 827-840(2019), https://doi.org/10.1056/NEJMoa1905230.
11. T. N. Kakuda, *Clin Ther*, **22(6),** 685-708(2000), https://doi.org/10.1016/S0149-2918(00)90004-3.
12. P. T. Giri, A. R. Chabukswar, S. C. Jagdale & S. A. Chindhe, *Int J App Pharm*, **17(4),** 241–253(2025), https://doi.org/10.22159/ijap.2025v17i4.54506.
13. L. Dickinson, S. Khoo, D. Back, *Antiviral Res*, **85(1),** 176-89(2010), https://doi.org/10.1016/j.antiviral.2009.07.017.
14. Z. Desta, T. Saussele, B. Ward, J. Blievernicht, L. Li, K. Klein, et al., *Pharmacogenomics*, **8(6),** 547–558(2007), https://doi.org/10.2217/14622416.8.6.547.
15. Y. Zhang, M. Huo, J. Zhou, S. Xie, P. K. Solver, *Comput Methods Programs Biomed*, **99(3),** 306-14(2010), https://doi.org/10.1016/j.cmpb.2010.01.007.
16. M. Blessy, R. D. Patel, P. N. Prajapati, Y. K. Agrawal, *J Pharm Anal*. **4(3),** 159-165 (2014), https://doi.org/10.1016/j.jpha.2013.09.003.
17. Z. Shaikh, O. Gadhave, B. Salunke, S. Aher, H. Shewale, N. Nagare and M. R. N. Shaikh, *Rasayan J. Chem*., **18(2),** 981-988 (2025). http://doi.org/10.31788/RJC.2025.1829240
18. D. S. Bhavsar, B. N. Patel, C. N. Patel, *Pharm Methods*. **3(2),** 73-8 (2012), https://doi.org/10.4103/2229-4708.103876.
19. L. Shribhumika, M. D. Ramgopal, *Res J Pharm Tech*, **17(3),** 1071-5(2024), https://doi.org/10.52711/0974-360X.2024.00167.
20. R. Godela, G. Sowjanaya, *Futur J Pharm Sci*, **6**, 9(2020), https://doi.org/10.1186/s43094-020-00026-0.