International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 11, Issue 4, pp: 33-39, 2026
https://doi.org/10.55126/ijzab.2026.v11.i04.005 **http://www.ijzab.com**

Research Article

METHOD DEVELOPMENT AND VALIDATION OF FEW ANTI-RETROVIRAL DRUGS ON BULK AND PHARMACEUTICAL DOSAGE FORM BY USING UV VISIBLE SPECTROSCOPY

[1]Nataraj Palaniyappan, *[2]Himabindu Erakasani, [3]Kannan Arumugam, [3]Vijayakumar Murugesan, [4]M. Ravisankar

[1]Novitium Pharma LLC, New Jersey, USA.
[2]Abiogenesis Clinpharm Private Limited, Punjagutta, Hyderabad-500082, Telangana, India
[3]School of Pharmacy, JSS Academy of Higher Education and Research, Mauritius
[4]Department of Pharmaceutical Chemistry, Srinivasan College of Pharmaceutical Science Trichy, Tamil Nadu, India

Article History: Received 17th April 2026; Accepted 19th May 2026; Published 1st July 2026

ABSTRACT

A simple, rapid, accurate, and cost-effective UV-Visible spectroscopic method was developed and validated for the simultaneous estimation of selected antiretroviral drugs, namely Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide, in bulk and pharmaceutical dosage forms. Two analytical methods were established based on absorbance maxima and iso-absorptive point principles. In Method I, Dolutegravir and Lamivudine were analyzed at 258 nm and 271 nm with an iso-absorptive point at 290 nm, while in Method II, Tenofovir Alafenamide and Emtricitabine were estimated at 263 nm and 281 nm, respectively. The developed methods obeyed Beer-Lambert's law within specified concentration ranges. Validation was carried out according to ICH guidelines including parameters such as linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The results demonstrated high correlation coefficients (>0.996), percentage recovery within acceptable limits, and %RSD values less than 2, indicating excellent precision and reliability. The proposed methods are suitable for routine quality control analysis of these drugs in pharmaceutical formulations due to their simplicity, sensitivity, and cost-effectiveness.

Keywords: UV-Visible spectroscopy, Dolutegravir, Lamivudine, Emtricitabine, Tenofovir Alafenamide.

INTRODUCTION

Pharmaceutical analysis plays a crucial role in ensuring the identity, safety, efficacy, and quality of drug products (Skoog, *et al.,* 2007). Among various analytical techniques, UV-Visible spectroscopy is widely used due to its simplicity, sensitivity, and cost-effectiveness (Ahuja, S & Scypinski, S, 2001). It is based on the absorption of electromagnetic radiation in the wavelength range of 200–800 nm, leading to electronic transitions in molecules (Willard, H. H., 1988). The quantitative estimation of drugs using this technique follows Beer-Lambert's law, which establishes a linear relationship between absorbance and concentration (Kar. A., 2005). Chromatographic techniques such as High - Performance Liquid Chromatography (HPLC) are also widely used; however, they require sophisticated instrumentation and higher operational costs (Dong, M. W., 2006). Therefore, UV spectroscopic methods remain preferable for routine analysis in quality control laboratories. Antiretroviral drugs such as Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide are widely used in the treatment of HIV infections. Accurate and reliable analytical methods are essential for their quality control and therapeutic monitoring. Several analytical methods including UV spectrophotometry and HPLC have been reported for individual and combined estimation of these drugs (Alnouti. Y *et al*., 2004).

*Corresponding Author: Himabindu Erakasani, Abiogenesis Clinpharm Private Limited, Punjagutta, Hyderabad-500082, Telangana, India, Email: himabindu@abiogenesisclinpharm.com.

The present study focuses on the development and validation of simple UV spectroscopic methods for simultaneous estimation of these drugs in bulk and dosage forms, following ICH guidelines to ensure accuracy, precision, and reproducibility Mandoli (DK *et al*., 2009).

MATERIALS AND METHODS

Materials and Instruments

A Shimadzu UV-Visible double beam spectrophotometer (PHARMASPEC-1800) with 1 cm quartz cells was used for absorbance measurements. Additional instruments included HPLC system (Shimadzu LC-20 AT), electronic balance, ultrasonicator, and pH meter (PV. Rajesh *et al.,* 2012). Pure drug samples of Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide were obtained as gift samples from pharmaceutical industries (Krishna reddy NV *et al.,* 2011).

Chemicals and Reagents

Methanol and distilled water were used as solvents. Other reagents included MBTH, ferric chloride, hydrochloric acid, sodium hydroxide, and NQS.

Method I: Simultaneous Estimation of Dolutegravir and Lamivudine (Baig M V *et al.,* 2001**)**

- λmax: 258 nm (Dolutegravir), 271 nm (Lamivudine)
- Iso-absorptive point: 290 nm
- Concentration range:
  Dolutegravir: 1–5 µg/ml
  Lamivudine: 6–30 µg/ml

Standard and sample solutions were prepared using methanol and diluted with water. Absorbance was measured at selected wavelengths, and concentrations were calculated using the absorbance ratio method.

Method II: Simultaneous Estimation of Emtricitabine and Tenofovir Alafenamide (Appalaraju S *et al.,* 2002**)**

- λmax: 263 nm (Tenofovir), 281 nm (Emtricitabine)
- Tablet formulation analyzed: TAFERO-EM

The powdered tablets were dissolved, filtered, and analyzed spectrophotometrically.

Method Validation

Validation was performed as per ICH guidelines (Shalini S *et al.,* 2009):

- Linearity
- Accuracy (Recovery studies at 80%, 100%, 120%)
- Precision (intra-day and inter-day)
- Robustness and Ruggedness
- LOD and LOQ

LOD and LOQ were calculated using:

$LOD = 3.3\sigma/S$
$LOQ = 10\sigma/S$

RESULTS AND DISCUSSION

Method I (Dolutegravir & Lamivudine) (Bahrami G *et al.,* 2005) The developed method showed excellent linearity with correlation coefficients: Dolutegravir: 0.9968–0.9981, Lamivudine: 0.9966–0.9987. The assay results indicated percentage purity close to 100%, confirming accuracy. %RSD values were less than 2%, indicating high precision. Recovery studies demonstrated values within acceptable limits (95–102%), confirming accuracy. LOD and LOQ values indicated high sensitivity of the method.

Table.1. Synthetic mixture for Dolutegravir & Lamivudine in 1:6 ratio Method Validation.

Ingredient	Quantity (mg)	Use
Dolutegravir	50	Anti-HIV drug
Lamivudine	300	Anti-HIV drug
MCC (Rolex chemical Industries, Mumbai)	10	Filler
Magnesium Sulphate (Thermo fisher scientific, Mumbai)	10	Lubricant
Mannitol	5	Sweetening agent
Sodium starch glycolate	15	Super disintegrant
PVP (SDFCL)	**10**	**Binder**

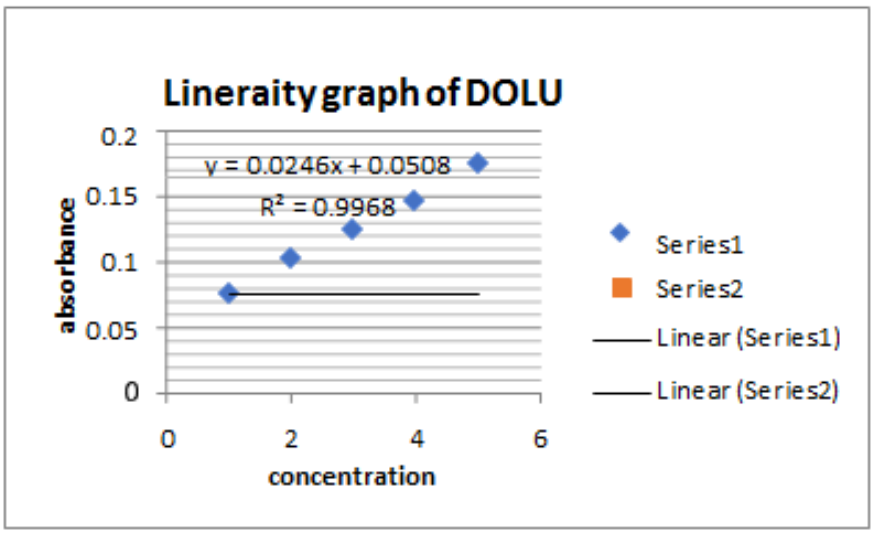

Figure 1. Linearity graph of Dolutegravir at 271 nm.

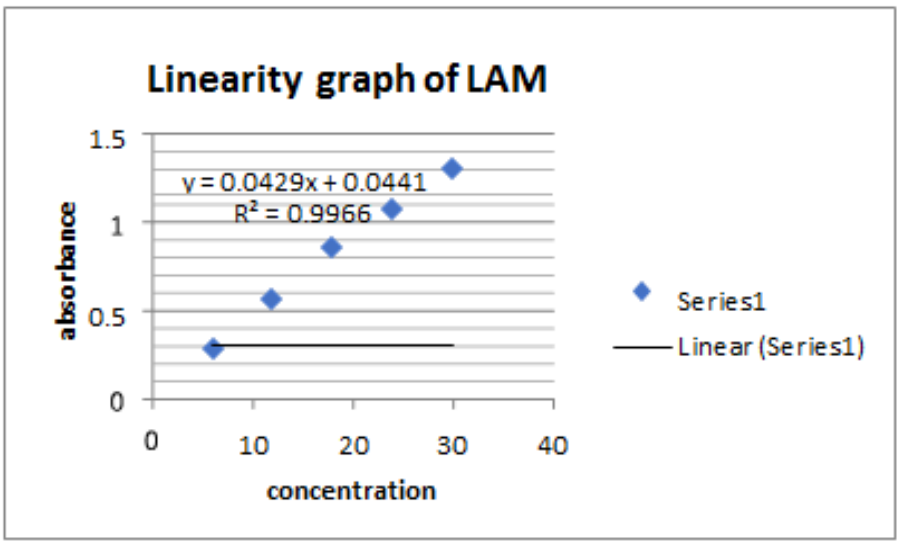

Figure 2. Linearity graph of Lamivudine at 271 nm.

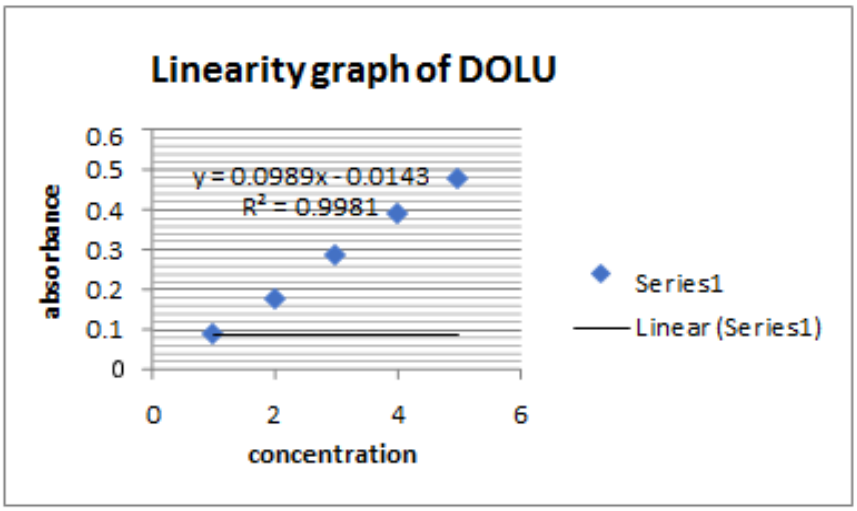

Figure 3. Linearity graph of Dolutegravir at 290 nm.

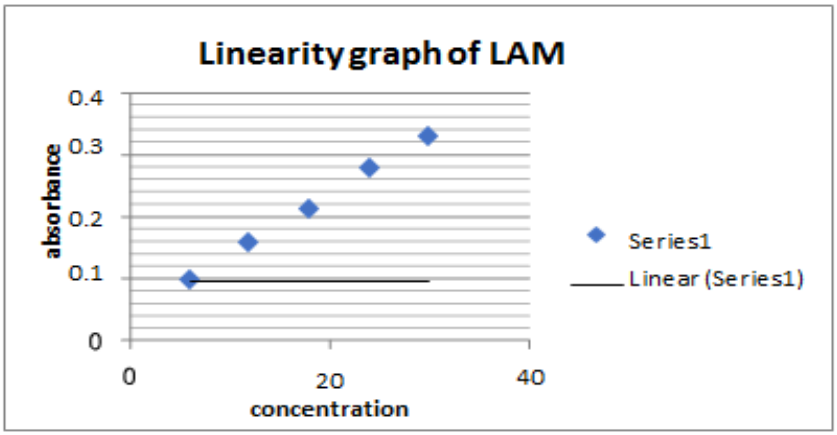

Figure 4. Linearity graph of Lamivudine at 290 nm.

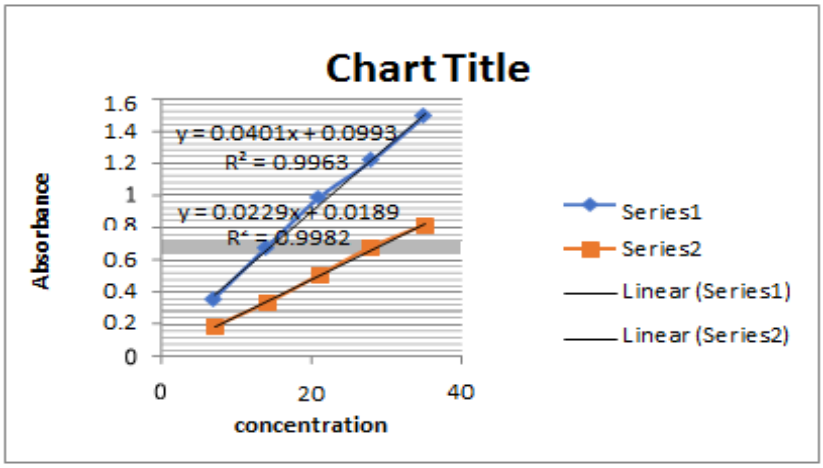

Figure 5. Linearity graph of mixture at isobestic point 290 nm.

Table 2. Linearity result mixture at 271 nm and 290 nm Accuracy.

Mixture at 271 nm		**Mixture at 290 nm**	
Conc (µg/ml)	Absorbance	Conc (µg/ml)	**Absorbance**
7	0.353	7	**0.183**
14	0.672	14	**0.328**
21	0.982	21	**0.500**
28	1.211	28	**0.675**
35	**1.486**	**35**	**0.810**

Table 3. Precision results.

Drug	**Amount taken (µg/ml)**	**Intra day** % content	% RSD	**Inter** % content	**Day** **% RSD**
		96.4		96.3	
DOLU	3	96.8	1.6	96.5	**1.6**
		96.6		96.7	
		98.9		98.6	
LAMI	**18**	98.8	**1.4**	98.5	**1.4**
		98.6		**98.8**	

Table 4. Results of LOD and LOQ.

Dolutegravir		**Lamivudine**	
LOD (µg/ml)	LOQ (µg/ml)	LOD (µg/ml)	**LOQ (µg/ml)**
0.350 (271nm)	1.03 (271nm)	2.07(271nm)	**6.29(271nm)**
0.263(290 nm)	**0.79(290 nm)**	**1.27 (290 nm)**	**3.8(290 nm)**

Method II (Emtricitabine & Tenofovir Alafenamide) (21-23). The assay of marketed formulation showed: Tenofovir Alafenamide: 96-96.8%. Emtricitabine: 97.5–97.6%. The developed method exhibited good linearity and precision with %RSD < 1%. The method was found to be robust and rugged under varied conditions.

Table 5. Linearity results Emtricitabine & Tenofovir Alafenamide

Tenofovir alafenamide		**Emtricitabine**	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
10.8	0.515	86.4	0.293
11.2	0.660	89.6	0.376
11.6	0.749	92.8	0.438
12	0.831	96	0.563
12.4	0.931	99.2	0.678
12.8	1.033	102.4	0.746

Table 6. Results of accuracy studies.

Drug	**Theoretical % target level**	**Amount added (mg)**	**Amount recovered (mean ± SD mg) n=3**	**% Recovery**	**% RSD**
			24.2 ± 0.3	96.8	
			24 ± 0.3	96	
	50	6	24.1 ± 0.2	96.4	**0.4**
			24.2 ± 0.4	96.8	
			24 ± 0.5	96	
TEN	100	12	24.3 ± 0.3	97.2	**0.6**
			24.1 ± 0.4	96.4	
			24.3 ± 0.5	97.2	
	150	18	24.2 ± 0.3	96.8	**0.4**
			195.2 ± 0.5	97.6	
			195.1 ± 0.6	97.5	
	50	48	195.1 ± 0.4	97.5	**0.05**
			195 ± 0.6	97.5	
			195.2 ± 0.8	97.6	
	100	96	195.1 ± 0.7	97.5	**0.05**
			195.3 ± 0.4	97.6	
EMT			195.2 ± 0.7	97.6	
	150	**144**	**195 ± 0.5**	**97.5**	**0.05**

Table 7. Precision results.

Drug	**Amount taken (µg/ml)**	**Intra day**		**Inter**	**Day**
		% content	**% RSD**	**% content**	**% RSD**
		96.4		96.3	
DOLU	3	96.8	1.6	96.5	1.6
		96.6		96.7	
		98.9		98.6	
LAMI	18	98.8	1.4	98.5	1.4

	98.6	98.8

Table 8. Results of LOD and LOQ.

Tenofovir alafenamide		**Emtricitabine**	
LOD (µg/ml)	LOQ (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
0.234	0.710	2.25	6.83

The present study successfully developed and validated two UV-Visible spectroscopic methods for the simultaneous estimation of selected antiretroviral drugs in bulk and pharmaceutical dosage forms. The selection of appropriate wavelengths played a critical role in achieving specificity and accuracy. In Method I, Dolutegravir and Lamivudine exhibited maximum absorbance at 258 nm and 271 nm, respectively, with an iso-absorptive point at 290 nm, which enabled the application of the absorbance ratio method for simultaneous estimation. In Method II, Tenofovir Alafenamide and Emtricitabine showed distinct absorbance maxima at 263 nm and 281 nm, respectively, allowing their independent quantification without interference (Shantakumari *et al.,* 2007). The developed methods demonstrated excellent linearity within the selected concentration ranges, as evidenced by high correlation coefficients (>0.996), confirming adherence to Beer-Lambert's law. The accuracy of the methods was validated through recovery studies, which yielded results within acceptable limits (95–102%), indicating minimal interference from excipients. Precision studies, including intra-day and inter-day analyses, showed %RSD values less than 2%, (Akhilesh Vikram Singh *et al.,* 2011) reflecting high reproducibility and reliability of the methods. Furthermore, the low values of LOD & LOQ indicated high sensitivity, enabling detection and quantification of drugs even at low concentrations. Robustness and ruggedness studies confirmed that minor variations in experimental conditions, such as solvent changes and analyst variability, did not significantly affect the results (Dinesh Dhatkar *et al.,* 2017). The assay of marketed formulations showed percentage purity close to the labelled claim, demonstrating the applicability of the developed methods in real pharmaceutical samples. Overall, the results indicate that the proposed UV spectroscopic methods are efficient alternatives to more complex chromatographic techniques for routine quality control analysis (Yashpalsinh N Girase *et al.,* 2018).

CONCLUSION

The present study was undertaken to develop simple, rapid, accurate, and cost-effective UV-Visible spectroscopic methods for the simultaneous estimation of Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide in bulk and pharmaceutical dosage forms. Two analytical methods were successfully developed based on absorbance maxima and iso-absorptive point principles. Both methods obeyed Beer-Lambert's law over the selected concentration ranges and demonstrated excellent linearity, accuracy, and precision. Validation of the developed methods was carried out in accordance with ICH guidelines, and all parameters including linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ were found to be within acceptable limits. The percentage recovery values confirmed the accuracy of the methods, while low %RSD values indicated high precision and reproducibility. The assay results of synthetic mixtures and marketed formulations were consistent with labeled claims, confirming the reliability of the methods. In conclusion, the developed UV-Visible spectroscopic methods are simple, economical, sensitive, and suitable for routine quality control analysis of antiretroviral drugs. These methods do not require sophisticated instrumentation or complex sample preparation, making them highly advantageous for use in pharmaceutical industries and research laboratories. Therefore, the proposed methods can be effectively employed for the estimation of these drugs in bulk and dosage forms, ensuring quality, safety, and efficacy of pharmaceutical products.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Abiogenesis Clinpharm Private Limited, Punjagutta, Hyderabad, Telangana, India for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Ahuja, S., & Scypinski, S. (2001). *Handbook of modern pharmaceutical analysis*. Academic Press.

Alnouti, Y., White, A. C., & Bartlett, G. M. (2004). Determination of lamivudine in plasma, amniotic fluid and rat tissues by liquid chromatography. *Journal of Chromatography B, 803*(2), 279–284.

Appalaraju, S., Karadi, A. B., Kamalapurakar, G. S., & Sarasambi, P. S. (2002). Spectrophotometric determination of lamivudine. *Asian Journal of Chemistry, 14*(1), 475–478.

Bahrami, G., Mirzaeei, S., Kiani, A., & Mohammadi, B. (2005). High-performance liquid chromatographic determination of lamivudine in human serum using liquid-liquid extraction: Application to pharmacokinetic studies. *Journal of Chromatography B, 823*(2), 213–217.

Baig, M. V., Kapse, G. S., & Raju, S. A. (2001). Spectrophotometric determination of lamivudine. *Asian Journal of Chemistry, 13*(1), 185–189.

Dhatkar, D., Ahmed, S., & Shastry, V. M. (2017). Development and validation of UV-visible spectrophotometric method for estimation of emtricitabine and tenofovir in bulk and dosage form. *International Journal of Pharmacy and Pharmaceutical Research, 4*(3), 74–84.

Dong, M. W. (2006). *Modern HPLC for practicing scientists*. Wiley.

ICH. (2005). *Validation of analytical procedures: Text and methodology Q2(R1)*. International Conference on Harmonisation.

Kar, A. (2005). *Pharmaceutical drug analysis*. New Age International.

Krishna Reddy, N. V., Phansi, R. S. C., & Ramesh Raju, R. (2011). New RP-HPLC method development for analysis and assay of lamivudine in formulation. *International Journal of Research in Pharmaceutical and Biomedical Sciences, 2*(1), 220–223.

Mandoli, D. K., Tyagi, P. K., Rai, V. K., Dey, S., Ashada, R. K., & Mohanraj, P. (2009). Method development and validation of RP-HPLC in the application of in vitro dissolution study of lamivudine in bulk drug and tablet formulation. *Journal of Chemical and Pharmaceutical Research, 1*(1), 286–296.

Rajesh, P. V., Karunasree, C. P., Dharmamoorthy, G., Padmini, K., & Sudheer, C. H. (2012). Development and partial validation of the lamivudine drug in bulk and solid dosage form by UV-spectroscopy. *International Journal of Pharmaceutical Development and Technology, 2*, 15–19.

Shalini, S., Shanooja, V. P., Jameel, S. A., Harilal, K. K., Rajak, H., & Ravichandran, V. (2009). Application of UV-spectrophotometric methods for estimation of lamivudine in tablets. *Digest Journal of Nanomaterials and Biostructures, 4*(2), 375–380.

Shantakumari, A., Prakash, K., Nagojit, K. E. V., & Rao, M. E. B. (2007). RP-HPLC method for the estimation of lamivudine in bulk and pharmaceutical dosage forms. *Asian Journal of Chemistry, 19*(4), 2642–2646.

Singh, A. V., Nath, L. K., & Pani, N. R. (2011). Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. *Journal of Pharmaceutical Analysis, 1*(4), 251–257.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2007). *Principles of instrumental analysis* (6th ed.). Thomson Higher Education.

Willard, H. H., Merritt, L. L., Dean, J. A., & Settle, F. A. (1988). *Instrumental methods of analysis* (7th ed.). CBS Publishers.

Yashpalsinh, N. G., Rao, S., & Soni, D. (2018). Development and validation of chiral RP-HPLC method for quantification of optical isomers in dolutegravir sodium. *Scholars Research Library, 10*(9), 90–100.