Journal of Pharmaceutical and Biological Research
ISSN: 2347-8330 | CODEN (USA): IJCPNH | Publisher: Pharma Research Library
Home Page: https://pharmaresearchlibrary.org/journals/index.php/jpbr
DOI: https://doi.org/10.30904/j.jpbr.2025.4788
J. Pharm. Bio. Res., 13(1), 2025: 15-20

# Method development and validation of few anti-retroviral drugs in bulk and pharmaceutical dosage form by using UV visible spectroscopy

N. Jayasree[1], P. Varshitha[1], G. Vineela[1], E. Supraja[1], D. Deepthi[1], N. Hari Chandana*[2], Y. Prapurnachandra[3]

[1]B.Pharm 4th Year, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore-524346, A.P, India
[2]Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.
[3]Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

**A B S T R A C T**

A comprehensive study was undertaken to develop and validate multiple analytical methods for the simultaneous and individual estimation of antiviral drugs Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide in bulk and pharmaceutical dosage forms. The methods included UV-Visible spectrophotometry (Methods 1–4), reversed-phase high-performance liquid chromatography (RP-HPLC; Methods 5–6), and liquid chromatography-tandem mass spectrometry (LC-MS/MS; Methods 7–8). Spectrophotometric methods, including Q-absorbance ratio and colorimetric techniques, demonstrated simplicity, precision, and cost-effectiveness. Absorbance maxima and is absorptive points were strategically utilized for drug quantification with acceptable limits of detection and quantification, making them suitable for routine quality control. RP-HPLC methods, employing O-Phosphoric acid and acetic acid buffer systems, achieved rapid separation with excellent linearity, accuracy, and robustness. LC-MS/MS techniques provided the highest sensitivity and selectivity, with ng/ml detection limits, minimal sample preparation, and high throughput analysis capabilities. Each method was validated as per ICH guidelines, ensuring precision, linearity, accuracy, and ruggedness. The developed methodologies offer scalable, reliable tools for routine analysis, quality assurance, and regulatory compliance in pharmaceutical environments, with method selection tailored to resource availability and sensitivity requirements.

**Keywords:** Antiviral drugs, Dolutegravir, Lamivudine, Emtricitabine, Tenofovir, Alafenamide, RP-HPLC, LC-MS/MS

**A R T I C L E I N F O**

**Corresponding Author**
**N. Hari Chandana**
Assistant Professor, Department of Pharmaceutical Analysis
Ratnam Institute of Pharmacy,
Pidathapolur (V), Muthukur (M), Nellore-524346, A.P, India.

**Article History**
**Received** : 04 March 2025
**Revised** : 25 March 2025
**Accepted** : 09 April 2025
**Published** : 29 April 2025



***Citation:*** *N. Jayasree, et al. Method development and validation of few anti-retroviral drugs in bulk and pharmaceutical dosage form by using UV visible spectroscopy. J. Pharm. Bio. Res., 2025, 13(1): 15-20.*

**CONTENTS**



## 1. Introduction

Antiretroviral therapy (ART) plays a pivotal role in the management and treatment of Human Immunodeficiency Virus (HIV) infection. Among the broad spectrum of antiretroviral agents, Dolutegravir, Lamivudine, Emtricitabine, and Tenofovir Alafenamide are notable for their efficacy, safety, and specific mechanisms of action that target viral replication at different stages. These drugs are primarily administered orally and are classified as antiviral agents with activity against HIV, and in some cases, chronic hepatitis B virus (HBV) infections. Dolutegravir ($C_{40}H_{19}F_2N_3O_5$, MW: 419.4 g/mol) is an integrase strand transfer inhibitor (INSTI) that prevents the

integration of viral DNA into the host genome, a crucial step in the HIV replication cycle. It has a long half-life of approximately 14 hours, exhibits high plasma protein binding (98.9%), and is primarily metabolized by UGT1A1 with contributions from CYP3A4. The drug is excreted through both renal and fecal routes and demonstrates potent antiviral activity at nano molar concentrations.

Lamivudine ($C_8H_{11}N_3O_3S$, MW: 229.26g/mol) is a nucleoside reverse transcriptase inhibitor (NRTI) that is intracellularly phosphorylated to its active form, lamivudine triphosphate. This metabolite causes viral DNA chain termination, effectively halting replication. Lamivudine has a half-life of 5 to 7 hours and is excreted largely unchanged via the renal pathway.

Emtricitabine ($C_8H_{10}FN_3O_3S$, MW: 247.24 g/mol), structurally similar to Lamivudine, is also a cytidine analog and NRTI. It inhibits HIV-1 reverse transcriptase after phosphorylation to its active triphosphate form, causing premature chain termination. It has a half-life of around 10 hours and is excreted renally.

Tenofovir Alafenamide (MW: 476.47 g/mol) is a prodrug of Tenofovir, a nucleotide analog reverse transcriptase inhibitor (NtRTI). Once converted in the body, it inhibits viral replication by mimicking natural nucleotides and terminating the viral DNA chain. It has a short plasma half-life of 0.51 hours but delivers high intracellular concentrations of the active drug. Collectively, these agents form the backbone of various combination therapies for the effective suppression of HIV-1 and HBV, improving patient outcomes and reducing viral resistance.

## 2. Methodology

### Drug sample

- Pure drug sample of Emtricitabine and Tenofovir alafenamide was kindly supplied as a gift sample by Mylan Laboratories Ltd., Hyderabad.
- Pure drug sample of Dolutegravir and Lamivudine was kindly supplied as a gift sample by Mylan Laboratories Ltd., Hyderabad and Hetero Lab, Hyderabad.

### Formulation

- The Tablets used for the analysis was TAFERO-EM manufactured by Hetero Labs Ltd, Himachal Pradesh, India, containing Emtricitabine 200 mg and Tenofovir Alafenamide 25 mg per tablet were procures from the market.
- Dolutegravir (50mg) tablet [INSTGRA] were produced from market (Emcure Pharmaceuticals)
- Lamivudine (100mg) tablet [LAMIVIR] were produced from market (Cipla pharmaceuticals)

### Chemicals and Reagents:

- Deionized water
- 0-Phosphoric acid (Thermo fisher Scientific India Pvt. Ltd, Mumbai) Water HPLC Grade (Thermo Fisher Scientific India Pvt. Ltd)
- HPLC Graded Acetic acid (Hi Media Laboratories Pvt. Ltd)
- **Reagents:** All other reagents used were of analytical grade were obtained from S.D fine chemicals Mumbai.
- **MBTH reagent:** 0.5% MBTH reagent was prepared by accurately weighing 0.5gm and dissolving it in 1% HCl.
- **Ferric chloride:** 1% (1gm of ferric chloride was accurately weighed and dissolved in 100ml distilled water) – Nice Chemicals

**Hydrochloric acid:**NQS (1,2-naphthaquinone-4-sulphonate sodium) NaOH (Sodium hydroxide) Methanol

- Methanol HPLC Grade Formic acid Ammonium formate

### Selection of solvent

Selection of a suitable solvent is influenced by the wavelength expected to be studied. The solvents thus used for the present study are, Methanol – Freely solubility of the drug Distilled water – For dilution of the stock

### Selection of wavelength

A 10mg of standard Dolutegravir and Lamivudine were weighed and transferred to 100ml separate volumetric flask, made upto volume with water contains 100 µg/ml of Dolutegravir and Lamivudine. The solution was scanned in the range of 200-400nm and the maximum absorbance was noted at 258 nm for DOLU (Fig.1) and 271 nm for LAMI (Fig.2) against water as blank and the iso- absoptive point was noted at 290nm.

### Preparation of standard stock solution

An accurately weighed synthetic mixture equivalent to 5mg of Dolutegravir and 30mg of Lamivudine was transferred into 50 ml standard flask. Dissolve the content in little amount of methanol and was sonicated using ultra-sonicator for 5 minutes and made upto volume with water. Appropriate aliquots within the Beer‟s Law limit (100µg/ml DOLU and 600µg/ml LAMI) were analyzed by proposed method.

### Linearity

From standard solutions, 0.1 ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml of aliquots are pipette into 10ml volumetric flask and made up to mark with water to give concentrations of 1-5 µg/ml of Dolutegravir and 6-30 µg/ml of Lamivudine and linearity was measured by regression analysis.

### Accuracy

The accuracy of the method was determined using recovery analysis. A known quantity of mixed pure drug was added to the accurately weighed synthetic mixture at 80%, 100%, and 120% levels. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation was calculated.

### Precision

From synthetic mixture, particular concentration level 3 µg/ml of DOLU and 18 µg/ml Lamivudine were prepared and analyzed in three replicates during the same day (intra-day) and on three consecutive days (inter-day). And the percentage relative standard deviation was also calculated.

### Robustness

Robustness of the method was estimated by introducing change in the solvent system from water to methanol.

Ruggedness

Ruggedness was determined by performing analysis of the synthetic mixture following the recommended procedures by three different analysts.

**Detection and Quantification Limit:**

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the intercept standard deviation and the curve slope.

LOD =3.3σ/S LOQ =10σ/S

## 3. Results and Discussion

**Selection of wavelength**

Dolutegravir and Lamivudine stock solution was prepared separately and the maximum absorbance was noted at 258nm for DOLU (Fig.1) and 271 nm for LAMI (Fig.2) against water as blank. The overlay spectrums of the mixture of the drug were given in (Fig. 3)

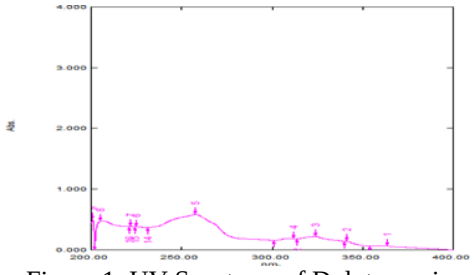

Figure 1: UV Spectrum of Dolutegravir

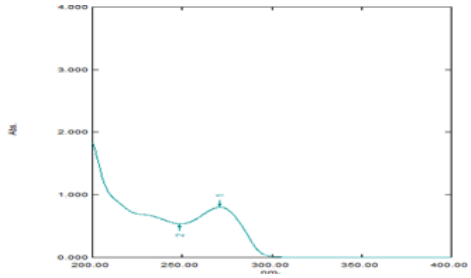

Figure 2: UV Spectrum of Lamivudine

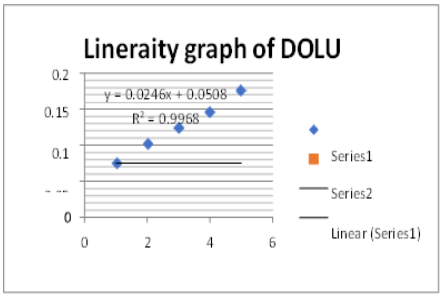


Figure 4: Linearity graph of Dolutegravir at 271 nm

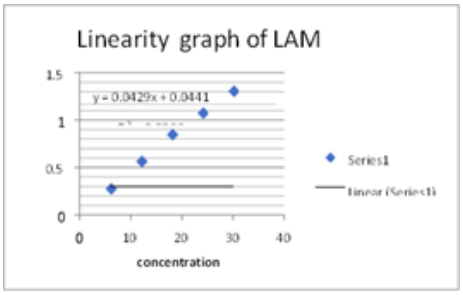


Figure 5: Linearity graph of Lamivudine at 271 nm

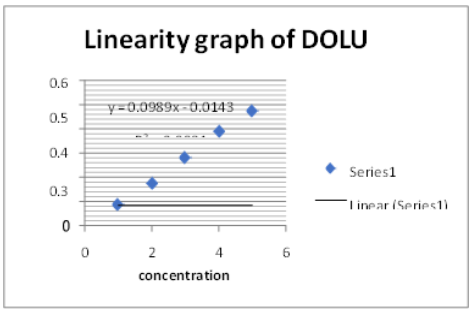


Figure 6: Linearity graph of Dolutegravir at 290 nm

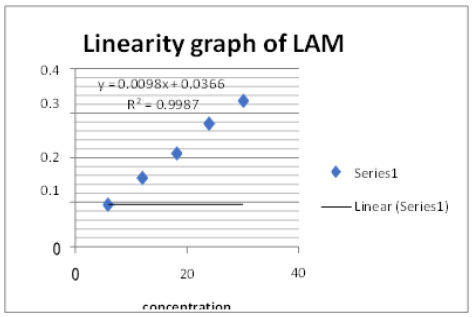


Figure 7: Linearity graph of Lamivudine at 290 nm

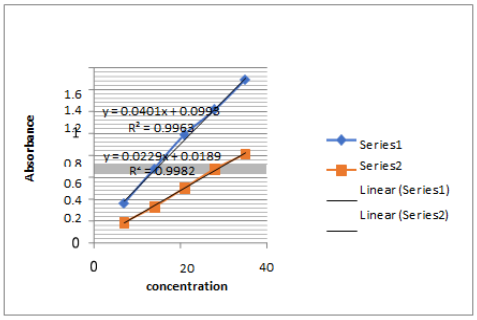


Figure 8: Linearity graph of mixture at isobestic point 290 nm

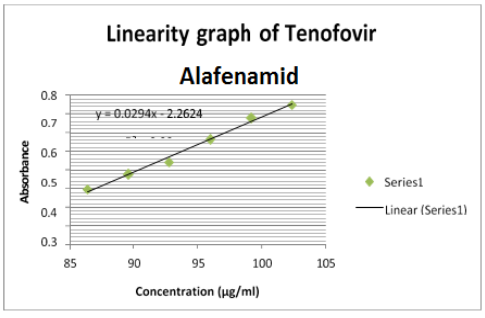


Figure 13: Linearity graph of Tenofovir Alafenamide

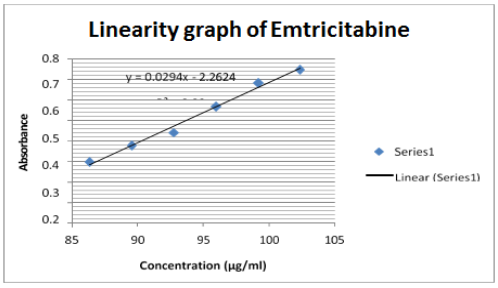


Figure 14: Linearity graph of Emtricitabine

**Table: 1** Design matrix summarizing the levels of 13runs of CCD for optimization of THC loaded LNs.

| **Dolutegravir** | | **Lamivudine** | |
|---|---|---|---|
| **Conc (µg/ml)** | **Absorbance** | **Conc (µg/ml)** | **Absorbance** |
| 1 | 0.075 | 6 | 0.278 |
| 2 | 0.102 | 12 | 0.565 |
| 3 | 0.124 | 18 | 0.850 |
| 4 | 0.146 | 24 | 1.076 |
| 5 | 0.176 | 30 | 1.308 |

Table 4: Linearity result of Dolutegravir and Lamivudine at 290 nm

| **Dolutegravir** | | **Lamivudine** | |
|---|---|---|---|
| **Conc (µg/ml)** | **Absorbance** | **Conc (µg/ml)** | **Absorbance** |
| 1 | 0.088 | 6 | 0.095 |
| 2 | 0.176 | 12 | 0.155 |
| 3 | 0.282 | 18 | 0.210 |
| 4 | 0.391 | 24 | 0.277 |
| 5 | 0.475 | 30 | 0.328 |

Table 6: Results of accuracy studies

| **Drug** | **Theoretical % target level** | **mount added (µg)** | **Amount covered (mean ± SD, µg)** n=3 | **% Recovery** | **% RSD** |
|---|---|---|---|---|---|
| DOLU | 80 | 2.4 | 5.2 ± 0.6 | 96.3 % | 1.7 |
| | 100 | 3 | 6.1 ± 0.3 | 101.66% | |
| | 120 | 3.6 | 6.7 ± 0.3 | 101.51% | |
| LAMI | 80 | 14.4 | 30.8 ± 0.1 | 95.06% | 1.8 |
| | 100 | 18 | 35.9 ± 0.5 | 99.72% | |
| | 120 | 21.6 | 40.10 ± 0.2 | 101.26% | |

Table 8: Robustness results

| **Drug** | **Amount taken (µg/ml)** | **Amount recovered (mean ± SD mg)** n=3 | **% Content** | **% RSD** |
|---|---|---|---|---|
| DOLU | 3 | 3.1 ± 0.4 | 103.3 | 1.8 |
| | | 3.2 ± 0.2 | 106 | |
| | | 3.2 ± 0.2 | 106 | |
| LAM | 18 | 18.6 ± 0.5 | 103.3 | 1.7 |
| | | 18.3 ± 0.7 | 101.6 | |
| | | 18.5 ± 0.5 | 102.7 | |

Table 9: Ruggedness results

| Drug | Analyst | Amount taken (µg/ml) | Amount recovered (mean± SD mg) n=3 | % Content | % RSD |
|---|---|---|---|---|---|
| DOLU | Analyst I | 3 | 3.1 ± 0.3 | 103.3 | 1.7 |
| | Analyst II | | 3.1 ± 0.5 | 103.3 | |
| | Analyst III | | 3.2 ± 0.2 | 106 | |
| LAMI | Analyst I | 18 | 18.2 ± 0.6 | 101 | 1.6 |
| | Analyst II | | 18.3 ± 0.6 | 102.2 | |
| | Analyst III | | 18.3 ± 0.5 | 101.6 | |

Table 10: 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) |

## 4. Conclusion

A simple, rapid, accurate and economical method has been developed for the simultaneous estimation of Dolutegravir and Lamivudine in synthetic mixture by using Q–absorbance ratio method. Absorbance ratio method for the ratio of absorbance at two selected wavelength, one which is an iso -absorptive point and other being λ max of one of the two components. Dolutegravir and Lamivudine showed an iso - absorptive point at 290 nm. The second wavelength used was 271 nm which is λ max of Lamivudine. The linearity of the method was found to be in the range of 1-5 µg /ml of Dolutegravir and 6- 30 µg/ml of Lamivudine. The concentration of the drugs was determined by using ratio of absorbance at iso - absorptive point and at the λ max of Lamivudine. The limit of detection of Dolutegravir was found to be 0.350µg/ml at 271 nm and 0.263µg/ml at 290nm. The limit of quantification of Dolutegravir was found to be 1.03µg/ml at 271 nm and 0.79µg/ml at 290 nm. The limit of detection of Lamivudine was found to be 2.07 µg/ml at 271nm and 1.27µg/ml at 290 nm. The Limit of quantification was found to be 6.29µg/ml at 271nm and 3.8µg/ml at 290 nm. Hence the method can be applied for routine quality control of the drugs. The UV-Visible Spectrophotometric method developed for the simultaneous estimation of the drug combination was found to be simple and economical as the method only uses distilled water and methanol as the solvent system. And also, the method was found accurate, precise, linear and rugged, so the newly developed method can be used for the routine analysis of the drug combination in combined dosage form.

## 5. References


[1] Douglas A Skoog, F. James Holler, Stanley R Crouch. Principles of Instrumental analysis, Sixth edition, Thomsons Higher Education, 1-3.

[2] Satinder Ahuja, Stephen Scypinski. Handbook of modern pharmaceutical analysis III, Academia press, 1-10.

[3] Hobart H Willard, Lynne L Merritt, John A Dean, Frank A Settle. Instrumental Method of Analysis 7th edition, CBS Publishers and Distributors, 1-3, 540- 608.

[4] Dr.M.R.Rajeswary. Biophysical and Biomedical techniques – Spectroscopic Techniques, 2-7.

[5] Ashutosh Kar. Pharmaceutical Drug Analysis, New Age International (p) limited, New Delhi, 301-303.

[6] G. Gaugliz and T. Vo-Dinh. Handbook of Spectroscopy, WILEY –VCH Verlag GmbH & Co.KGaA, 100-101.

[7] Peter A Sewel & Brian Clarke. Chromatography Separation, 1-7.

[8] Satinder Ahuja. Chromatography and Separation Science, Volume 4, 102- 111.

[9] Jackes Cazes. Analytical instrumentation Method, Third edition, 687-713, 744-750.

[10] Michael.W.Dong. Modern HPLC for practicing Scientists, 17-96.

[11] Skoog D.A, West D.M, Holler F.J (1996). Fundamentals of analytical chemistry. (8th edition), Fort Worth: Saunders College Publishers 12-63.

[12] Validation of compedial assays-Guidelines Pharmacopoeial Conversation, Rochvilie, MD, 1985, 25-36.

[13] Nishnt Toomula, Arun Kumar, Satish Kumar D and Vijaya Shanti Bheemidi. Development and Validation of Analytical Methods for Pharmaceuticals, Analytical & Bioanalytical Techniques 2011, 2(5).

[14] A.Shantakumari ,K.Prakash, K.E.V.Nagojit and M.E.B.Rao ; RP-HPLC Method for the Estimation of Lamivudine in Bulk and Pharmaceutical Dosage Forms ; Asian Journal of Chemistry , Vol. 19,No. 4 (2007),2642- 2646

[15] Akhilesh Vikram Singh , Lila K. Nath and Nihar R. Pani; Development and Validation of Analytical Method for the Estimation of Lamivudine in Rabbit plasma; Journal of Pharmaceutical Analysis 2011;1(4):251-257

[16] Kalpesh V. Sonar, Prabodh Sapkale, Anil Jadav, Tushar Deshmukh, Swapnil Patil, Pallavi Murkute; International Journal of Current Pharmaceutical Research, Vol 9, Issue 6,2017

[17] K. Anand Kumar, K. Anusha, V. Kokiklavani, V.P. Sangeetha, R. Saranya, M. Jambulingam and J. Ramesh; Method Development and Validation of UV- Spectroscopic Method for the Determination of Lamivudine as an Active Pharmaceutical Ingredient and in Tablet dosage form; 2017, 05(04): 129-137

[18] Ragupathi. G and C. Parthiban; Method Development and Validation for the Determination of Lamivudine in Tablet Dosage Form by UPLC Method; International Journal of Pharmacy and Pharmaceutical Sciences Vol 5, Issue 4, 2013.

[19] Alnouti. Y, White AC and Bartlett GM; Determination of Lamivudine in Plasma, Amniotic fluid and Rat tissues by Liquid Chromatography; J Chromatogr B 2004;803: 279-284

[20] Mandoli DK, Tyagi PK, Rai VK, Dey S, Ashada RK and Mohan raj P; Method Development and Validation of RP-HPLC in the application of Invitro Dissolution study of Lamivudine in bulk drug and Tablet Formulation; Journal of Chemical and Pharmaceutical research.1(1), 2009, 286-296

[21] PV. Rajesh, CP Karunasree, G Dharmamoorthy, K Padmini and CH Sudheer; Development and Partial Validation of the Lamivudine drug inBulk and Solid Dosage form by UV-Spectroscopy; IJPDT 2012;2:15-9

[22] Krishna reddy NV, Phansi RSCh, and Ramesh raju R; New RP-HPLC Method Development for Analysis and Assay of Lamivudine in Formulation. International Journal of Research

in Pharmaceutical and Research in Pharmaceutical and Biomedical sciences 2011; 2(1): 220-223.

[23] Baig M V, Kapse G S and Raju S A; Spectrophotometric Determination of Lamivudine; Asian Journal of Chemistry,13(1), 2001,185-189

[24] Appalaraju S, Karadi A B, Kamalapurakar G S and Sarasambi P S. Spectrophotometric Determination of Lamivudine, Asian Journal of Chemistry; 14(1),2002,475-478

[25] Shalini S, Shanooja, V P, Jameel S A, Harilal K K, Rajak H and Ravichandran V. Application of UV-Spectrophotometric methods for Estimation of Lamivudine in Tablets. Digest Journal of Nanomaterials snd Biostructures, 4(2),2009, 375-60.

[26] Bahrami G, Mirzaeei S,Kiani A and Mohammadi B. High –Performance Liquid chromatographic Determination of Lamivudine in Human serum using Liquid-Liquid Extraction; Application to Pharmacokinetic studies, Journal of Chromatography B,823(2),2005,213-217

[27] Zheng jj., et al. "High-Performance Liquid Chromatographic assay for the Determination of Lamivudine in Human plasma". Journal of Chromatography B 761(2001):195-201

[28] Kano EK., et al." Determination of Lamivudine in Human plasma by HPLC and its use in Bioequivalence studies". International Journal of Pharmaceutics 297.1-2(2005):73-79

[29] C. josegnababu and G. Vijayakumar "Validate Spectrophotometric Estimation of Lamivudine in Pure and Tablet Dosage Form" International Journal of Chemtech Resaerch,2009,1(4),1372-1375

[30] N. M. D. Akram, M Umamahesh, A New Validated RP-HPLC Method for Detrmination Of Emtricitabine And Tenofovir AF In Its Bulk and Pharmaceutical Dosage Form, Journal of Chemical and Pharmaceutical Sciences, 2017 (10), 54-59.

[31] S.K Machinima, D. Ventaka Reddy, P. Soidulu, M. Varalakshmi, Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Emtricitabine, Tenofovir Alafenamide In Bulk and Their Combined Dosage Form, Journal of Chemical and Pharmaceutical Research, 2017, 9(9), 70-80.

[32] Bhushan P. Bedeguar, Moreshwar P. Mahajan, Sanjay D. Sawant. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Tenofovir Alafenamide And Emtricitabine In Bulk and Tablet Dosage Form, International Journal of ChemTech Research, 2017, 10 (5), 731- 739.

[33] Gandla Kumara Swamy, M. Rajkumar, K. Pranay, D. Sudheer Kumar. New Stability Indicating RP-HPLC Method for The Simultaneous Estimation of Tenofovir Alafenamide And Emtricitabine In Bulk and Tablet Combine Tablet Dosage Forms, Asian Journal of Pharmaceutical Analysi And Medicinal Chemistry, 2017, 5 (4), 142- 149.

[34] Benzil Dudekula, Dr. C. Ravichandran, Dr. C. Ramachandraiah, Dr. N. Devanna. Development and Validation Of RP-HPLC Method for The Simultaneous Estimation of Emtricitabine And Tenofovir Alafenamide In Bulk and Tablet Dosage Form, European Jpurnal Of Biomedical and Pharmaceutical Sciences, 2017, 4 (10), 663-668.