IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
June 2022 Vol.:24, Issue:3

Comprehensive Review on Various Analytical Methodologies for the Estimation of Lamivudine

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Santosh V. Gandhi*, Hanmant A. Bade, Manisha S. Jagtap, Shivani R. Sawarkar, Varsharani S. Pawar, Vinod V.Gaikwad

AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune-411001, India.

Submitted: 20 May 2022
Accepted: 25 May 2022
Published: 30 June 2022

Keywords: Lamivudine, HPLC, HPTLC, Analytical method.

ABSTRACT

Lamivudine belongs to the class of antireteroviral drugs which acts as a nucleoside reverse transcriptase inhibitor and used in the treatment of patients with HIV. It belongs to 3'-thia pyrimidine nucleosides. USFDA approved lamivudine in June 2001. The current review is mainly based on the analytical, bioanalytical method development approaches and various methods developed for the estimation of Lamivudine either in pharmaceutical dosage form or in bulk. Analytical techniques are best suited for the estimation of the drug and and have a great impact on qualitative as well as quantitative results. The presented methods may help in understanding the important parameters and the factors affecting the results. The current study is a compendium of several dose forms and analytical procedures for quantitative analysis of Lamivudine that have been described.

INTRODUCTION:

Lamivudine belongs to a group of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs) category which is mostly used for the treatment of patients with HIV.[1] It is a well-known antiretroviral drug focused on the treatment of HIV, AIDS, Hepatitis B Virus. Structurally Lamivudine is 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2-one. It is a synthetic nucleoside analog that gets phosphorylated to its active metabolites as lamivudine triphosphate and lamivudine monophosphate. This compound belongs to the class of organic compounds known as 3'-thia pyrimidine nucleosides.[2] Lamivudine is sold under various brand names with different dosage strength as 100mg, 150mg and 300mg. USFDA had approved lamivudine in June 2001.[3] Some study had also reported that the use of lamivudine, improves liver function and lessen the need for a liver transplant.[4-7] In the United States, about 5,000 deaths occur each year from hepatitis B virus (HBV)-related liver illness.[8] Lamivudine, a novel nucleoside analogue, is an effective inhibitor of hepatitis B viral replication with no clinically significant adverse effects.[9] Lamivudine, which was given before and after liver transplantation to avoid HBV graft reinfection, caused a full and long-lasting suppression of viral replication.[10] Various analytical methods are reported for the estimation of Lamivudine in bulk as well as dosage forms by using UV spectroscopy, HPLC, HPTLC, etc which are discussed in this paper.

METHODOLOGY:

An exhaustive literature search was undertaken utilizing science direct, Taylor and Francis, Springer, Google Scholar, and the Wiley database. During the literature search, the terms Lamivudine, analytical development, HPLC, UV, bioanalytical, validation, HPTLC, LCMS, HIV, and Hepatitis were employed. Articles that were found to be relevant were evaluated and presented in this paper.

Physicochemical Properties:

The structure of Lamivudine is shown in Fig. 1. Lamivudine appears as acicular or bipyrimidal crystals. It is water-soluble, sparingly soluble in methanol, and insoluble in acetone. Lamivudine has a pKa of 4.3, making it a weak base (protonation of the NH_2 group).[11]Its molecular weight is 229.26 g/mol, absorption maximum 271 nm[12] & melting point is 160-162˚C. [13]

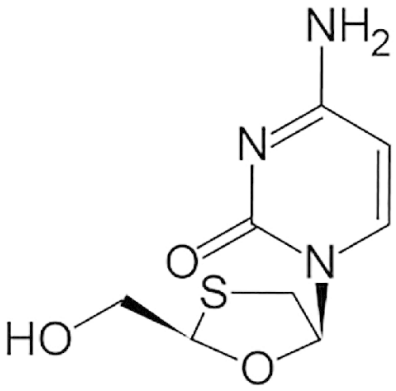

Fig. No. 1: Chemical structure of Lamivudine

Pharmacokinetic:

Lamivudine is rapidly absorbed, with C_{max} usually reaching in 0.5 to 1.5 hours after the oral administration. In adults and children, absolute bioavailability is at 82 and 68 percent, respectively. When lamivudine is given with food, the area under the serum drug concentration-time curve (AUC) does not change. Following intravenous injection, lamivudine is broadly dispersed in total body fluid, with a mean apparent volume of distribution (Vd) of roughly 1.3 L/kg. In persons with normal renal function, about 5% of the initial medication is metabolized to the trans-sulphoxide metabolite, which is pharmacologically inactive.[14]

Pharmacodynamic:

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) that stops the human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus from making DNA (HBV). Lamivudine can be phosphorylated to produce active metabolites that compete for viral DNA incorporation. Lamivudine metabolites function as a chain terminator of DNA synthesis by competitively inhibiting the activity of the HIV reverse transcriptase enzyme via DNA incorporation. Incorporated nucleoside analogues hinder the development of a 5' to 3' phosphodiester linkage, which is required for DNA chain elongation because they lack a 3'-OH group.[15]

Approved dosage forms of Lamivudine[16]:

Table 1 lists the commonly available dosage forms in the market, along with their brand names and manufacturers.

Table No. 1: Lamivudine dosage forms along with brand names

Sr. no	Brand Name	Manufacturer	Dosage form
1	Lamivir HBV	Cipla Ltd	Tablet
2	Hivir	Sain Medicaments Pvt Ltd	Tablet
3	Lamimat	Mylan Pharmaceuticals Pvt Ltd	Tablet
4	Lamidac	Zydus Cadila	Tablet
5	Heptavir	Hetero Drugs Ltd	Syrup
6	Hepitec	GSK Pharmaceuticals Ltd	Tablet
7	Epivir	GSK Pharmaceuticals Ltd	Oral solution
8	Shanvudin	Shantha Biotech	Tablet

The necessity of analytical and bioanalytical Methods:

Analytical approaches help determine the compositions in a formulation by employing advanced analytical tools to obtain both qualitative and quantitative data. Analytical and bioanalytical techniques are crucial in risk assessment and management. These techniques are useful to establish the identity, purity, physical characteristics and potency of the drug. It aids in the development of product-specific acceptability criteria as well as the consistency of results. Validation should show that the analytical approach is appropriate for the task at hand. The design of experiments is a powerful tool for characterization and validation of methods.[17] For pharmacokinetics, toxicokinetics, bioequivalence, and exposure-response studies, bioanalysis is utilised to provide a quantitative measure of the active medicine and/or its metabolite(s). In forensic and clinical toxicology, the dependability of analytical data is critical, since it is required for the interpretation of toxicological findings.[18]

Analytical method development by UV spectrophotometry[19-23]:

Sharma R. *et al.* developed a UV spectroscopic method for estimation of Lamivudine in a three-component tablet formulation. The analysis was carried out using the simultaneous estimation method and derivative spectroscopic method. **Rangapriya M. *et al.*** had developed a simple and cost effective UV spectroscopic method for the analysis of antireteroviral drug. They had showed that the solubility of drug is favourable in pH 6.8 phosphate buffer. The method report that the absorption maxima of Lamivudine at 271nm. **Deepali G. *et al.*** had reported a UV spectroscopic method for the assay of Lamivudine in the bulk and its tablet

dosage form. The analysis was carried out using spectroscopic grade Methanol as a solvent for the drug. They reported the absorption maxima of Lamivudine at 270 nm. **Gupta A. *et al.*** had developed a chemometric UV spectroscopic method for the estimation of Lamivudine in the formulation. The study describes the application of the multivariate method to obtain the spectroscopic data for the simultaneous estimation of drugs in combination. **Grangeiro Jr S *et al*.** had developed a method using UV spectroscopy and multivariate calibration is described for the simultaneous measurement of Lamivudine and Zidovudine. Table 2 lists the specifics of the instrument model, solvent, and wavelength that were used.

Table No. 2: Analytical method development using UV spectrophotometer

Sr no	Dosage form	Instrument model	Solvent/media	Wavelength	Reference
1	Tablet (Combination)	UV-Visible double beam spectrophotometer-1700 (Shimadzu)	Methanol:Water (50:50)	272	19
2	Tablet (Combination)	U.V. Spectrophotometer (Perkin Elmer Lamda-25).	Phosphate buffer pH 6.8	271	20
3	Bulk and Tablet	Elico SL-159 UV-visible spectrophotometer	Methanol	270	21
4	Tablet (Combination)	UV- Visible spectrophotometer model (UV-1800 Shimadzu	Methanol	272	22
5	Tablet (Combination)	Double beam UV-vis spectrometer, Shimadzu®, model UV-2401 PC	Methanol	270	23

Analytical method development by HPLC[24-29]:

Bhavsar *et al.* devised and validated a simple, accurate, and reliable reverse phase HPLC method for estimating Lamivudine in tablet dosage form. The separation of Lamivudine was performed on Kromasil C_{18} analytical column (150 × 4.6 mm, i.d., 5 µm) using methanol: 10mM phosphate buffer pH 5 in the ratio of 70:30 v/v, and detection was carried out at 254 nm. They reported the method with a flow rate of 1ml and the retention time of Lamivudine at 2.8 mins. **Rao *et al.*** reported the elution of Lamivudine at 2.8 mins with a 1ml/min flow rate. The method was developed and validated using Inertsil ODS-3V C_{18} (250 × 4.6 mm, 5µ) column running with gradient mobile phase, where mobile phase A was 0.05 M Phosphate buffer pH 6.2 and methanol 50:50% v/v and mobile phase B was 10ml OPA diluted with 1000 ml Acetonitrile. The developed method was validated according to ICH guidelines. **Noorbasha *et al.*** had developed a method for estimation of Lamivudine in Tablet dosage form on Inertsil ODS 3V (250 × 4.6 mm, 5 µm) with a mobile phase as phosphate buffer, pH 3.0 with acetonitrile and methanol in the proportion of 50:20:30% v/v/v. The estimation was carried out at 257nm and the retention time for Lamivudine was reported at 2.2mins. **Singh VD *et al.*** had developed a method for simultaneous estimation of the lamivudine and Raltegravir in a laboratory . Separaion was achieved on phenomenex C18 column (150×4.6mm id, 5µ particle size) and mobile phase was compose of 75% methanol : 15% acetonitrile : 10% (0.5Mm) phosphate buffer (pH 3.0), with flow rate 1.2ml/min at 254nm. **Tamilarasan S *et al*.** had developed precise, simple, economic and less time-consuming RP-HPLC method on Sun fire C8, (150 X 4.6mm, 5µm) column with Acetonitrile: Pottasium dihydrogen orthophosphate (55:45v/v) as mobile phase. **Mastanamma S.** had developed and validate RP-HPLC method for simultaneous estimation of Lamivudine,Tenofovir Alafenamide and Dolutegravir bulk and their combined dosage form.The method was established using Agilent (250 X 4.6 mm, I.d., 5µm) Column with mobile phase M Phosphate Buffer pH 6.2 : Acetonitrile 60:40 v/v and the detection was carried out at 260 nm. Reported HPLC methods for estimation of Lamivudine in bulk and tablet dosage form are summarised in the Table 3.

Table No. 3: Analytical method development using HPLC method

Sr No	Sample	Stationary phase/column	Mobile phase	Wavelength (nm)	Flow rate (ml/min)	RT (min)	Reference
1	Tablet (Combination)	Kromasil C_{18} analytical column (150 × 4.6 mm, i.d., 5 µm)	70:30 v/v methanol:10 mM phosphate buffer pH 5	254	1	2.8	24
2	Tablet (Combination)	Inertsil ODS-3V C_{18} (250 × 4.6 mm, 5µ).	A:0.05 M Phosphate buffer pH 6.2 and methanol 50:50% v/v and B: 10ml OPA diluted with 1000 ml Acetonitrile	260	1	2.8	25
3	Tablet (Combination)	Inertsil ODS 3V (250 × 4.6 mm, 5 µm)	phosphate buffer, pH 3.0:acetonitrile:methanol (50:20:30% v/v/v)	257	1	2.2	26
4	Tablet (Combination)	phenomenex C18 column (150 X 4.6 mm id, 5µ)	methanol: Acetonitril: (0.05mM) phosphate buffer (pH 3.0) 75:15:10% v/v/v	254	1.2	3.1	27
5	Bulk (Combination)	Sun fire C8,(150 X 4.6mm, 5µm)	Acetonitrile: Pottasium dihydrogen orthophosphate (55:45v/v)		1	3.1	28
6	Bulk and Tablet (Combination)	Agilent C18 (250 × 4.6 mm, i.d., 5 µm)	0.05M phosphate buffer pH 6.2 : acetonitrile 60:40 v/v	260	1	3.09	29

Analytical method development using HPTLC method[30-32]:

Gu Y *et al* studied and developed a suitable, sensitive stability-indicating method by using the HPTLC for the analysis of Lamivudine in the tablet dosage form. The stationary phase used in the experiment was Merck Premium Purity silica gel 60 F plates. Basic solubility studies and further trials helped to select the mobile phase as ethyl acetate, methanol, acetone and concentrated ammonium hydroxide in ratio of 30:7:3:1 v/v and the detection was carried out at 254nm. **Baig *et al*** had developed a stability-indicating HPTLC method for estimation of Lamivudine in tablet dosage form using Aluminium plates precoated with silica gel 60 F_{254} plates as stationary phase while mobile phase was selected based on the trials was Chloroform and methanol 9:1 v/v. Also, **Barsagade *et al*** had developed and validated a HPTLC method for estimation of Lamivudine along with zidovudine. The details of the method is summarized in the table 4.

Table No. 4: Analytical method development using HPTLC method

Sr No	Sample	Stationary phase/column	Mobile phase	Wavelength (nm)	Application rate µl/s	Scan speed mm/sec	Reference
1	Tablet	Merck Premium Purity silica gel 60 F plates	Ethyl acetate: Methanol: Acetone: Concentrated ammonium hydroxide (30:7:3:1 v/v)	254	-	10	30
2	Tablet	Aluminium plates precoated with silica gel 60 F_{254} plates	Chloroform: Methanol (9:1 v/v)	254	0.15	-	31
3	Tablet	HPTLC plates coated with silica gel 60 F254.	Toluene: Ethyl acetate: Methanol: Ammonia 6.5:2.5:1.5:1.5 (v/v)	276	5	20	32

Bio-analytical method development:[33-37]

Varma S *et al* had developed and validated a bioanalytical method for estimation of lamivudine and stativudine from human plasma by HPLC. The study was performed on Hypersil BDS 250mm × 4.6mm, 5µ column with mobile phase consisting ammonium acetate buffer and methanol in ratio 93:7 % v/v and detection was carried out at 270 nm. **Singh A V *et al*** had reported a validated method for estimation of Lamivudine in rabbit plasma. They demonstrated the reliable method on Hypersil BDS C18 (250mm × 4.6mm, 5µ) with mobile phase 0.25% Triethylamine buffer (pH 3.0): Acetonitrile (70:30 %v/v) and detection was carried out at 256nm. **Marakatham S *et al*** had developed and validated a bioanalytical method for estimation of Doravirine, lamavudine and tenofovir disoproxil fumarate from human plasma by HPLC using Phenomenex C18 (150mm x 4.6mm,5 µ) with mobile phase 0.01 N potassium dihydrogen phosphate (pH 3.5): Acetonitrile (60:40v/v) and detection was carried out at 277 nm. **Alnouti Y *et al*** had developed and validated(LC/MS/MS) method for the simultaneous determination of zidovudine and lamivudine in rat plasma, amniotic fluid, placental, and fetal tissues. Chromatography was performed by using a C18 column (5 µm, 150 x 3.9 mm i.d) with mobile phase 30 % methanol and 7.5 mM ammonium acetate (pH 6.5)**. Malm M. *et al*** had developed and validated bioanalytical method for the determination of lamivudine, zidovudine, and nevirapine in 100 µL capillary blood applied onto sampling paper has been by reversed-phase gradient liquid chromatography with UV detection. Separation was performed on a Zorbax SB C8 (250 × 4.6 mm) column with a twostep gradient: (i) methanol–0.05 mol/L acetic acid:sodium acetate buffer (pH 3.95, 15:85 v/v) and (ii) methanol–0.05 mol/L acetic acid:sodium acetate buffer (pH 3.95, 50:50 v/v) detection was performed at 260 nm. The details of above method is summarized in the below table 5.

Table No. 5: Bio-analytical validated methods

Sr No	Sample	Stationary phase/column	Mobile phase	Wavelength (nm)	Reference
1	Human Plasma	Hypersil BDS 250mm × 4.6mm, 5µ	Ammonium acetate buffer and methanol in ratio 93:7 % v/v	270	33
2	Rabbit plasma	Hypersil BDS C18 (250mm × 4.6mm, 5µ)	0.25% Triethylamine buffer (pH 3.0): acetonitrile (70:30, v/v)	256	34
3	Human Plasma	Phenomenex C18 (150mm x 4.6mm, 5µ)	0.01N Potassium dihydrogen phosphate (pH 3.5): Acetonitrile (60:40% v/v)	277	35
4	Rat Plasma (LC-MS)	C18 column (5 mm, 150 3.9 mm i.d)	30% methanol and 7.5 mM ammonium acetate (pH 6.5)	-	36
5	Blood	Zorbax SB C8 (250 × 4.6 mm)	(i) methanol–0.05 mol/L acetic acid:sodium acetate buffer (pH 3.95, 15:85 v/v) and (ii) methanol–0.05 mol/L acetic acid:sodium acetate buffer (pH 3.95, 50:50 v/v)	260	37

CONCLUSION:

This review focus on the numerous analytical and bioanalytical methods used to estimate Lamivudine in various formulations as well as in the bulk form of the pharmaceuticals. UV spectrophotometry, HPLC, HPTLC, RP-HPLC, etc. are some of the analytical and bioanalytical methods that the researchers have worked on. All of the established analytical

procedures are sensitive, repeatable, and precise. The purpose of a literature review is to gather and consolidate information on various analytical instrumental procedures. Such information might be useful in the development of new analytical methods.

CONFLICT OF INTEREST: Authors report no conflict of interest concerning this review article.

FUNDING STATEMENT: The presented work is not funded by any funding agency.

ACKNOWLEDGMENT: The authors are thankful to the Principal and management, of AISSM's College of Pharmacy, Pune, Maharashtra, for their kind help and suggestion. The authors are also thankful to the informants for sharing valuable information.

REFERENCES:

1. Medline Drug Information - Lamivudine. https://medlineplus.gov/druginfo/meds/a696011.html [Cited on 11th Oct 2021].
2. National Library of Information, Pubchem: https://pubchem.ncbi.nlm.nih.gov/compound/Lamivudine[Cited on 24th Oct 2021].
3. USFDA Drug approval package, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/20-564s012_20-596s013_Epivir.cfm [Cited on 24th Oct 2021].
4. Villeneuve J, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, *et al.* Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207-10.
5. Yao Fy, Bass NM. Lamivudine treatment in patients with severely decompensated cirrhosis due to replicating hepatitis B infection. J Hepatol 2000; 33:301-07.
6. Kapoor D, Guptan RC, Wakil S, Kazim SN, Kaul R, Agarwal SR, *et al*. Beneficial effects of lamivudine in hepatitis B virus related decompensated cirrhosis. J Hepatol 2000; 33:308-12.
7. Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: A comparative study using a matched, untreated cohort. Hepatology 2001; 34:411-16.
8. Fontana RJ, Keeffe EB, Carey W, Fried M, Reddy R, Kowdley KV, Soldevila-Pico C, McClure LA, Lok AS, National Institutes of Health Hepatitis B Virus Orthotopic Liver Transplantation Study Group. Effect of lamivudine treatment on survival of 309 North American patients awaiting liver transplantation for chronic hepatitis B. Liver Transplantation. 2002 May 1;8(5):433-39.
9. Honkoop P, De Man RA, Scholte HR, Zondervan PE, Van Den Berg JW, Rademakers LH, Schalm SW. Effect of lamivudine on morphology and function of mitochondria in patients with chronic hepatitis B. Hepatology. 1997 Jul; 26(1):211-15.
10. Ben-Ari Z, Shmueli D, Mor E, Shapira Z, Tur-Kaspa R. Beneficial effect of lamivudine in recurrent hepatitis B after liver transplantation. Transplantation. 1997 Feb 15;63(3):393-96.
11. Strauch S, Jantratid E, Dressman JB, Junginger HE, Kopp S, Midha KK, Shah VP, Stavchansky S, Barends DM. COMMENTARY: biowaiver monographs for immediate release solid oral dosage forms: lamivudine. Journal of pharmaceutical sciences. 2011 Jun 1; 100(6):2054-63.
12. Haque MA, Mounika K, Bakshi V. Development and Validation of UV Spectrophotometric Method for Estimation of Lamivudine in Bulk and Tablet Dosage Form. 2018, 10(5): 108-111.
13. Lamivudine WH. Final text for addition to The International Pharmacopoeia. QAS/05.123/FINAL) 2006: 1–7.
14. Johnson MA, Moore KH, Yuen GJ, Bye A, Pakes GE. Clinical pharmacokinetics of lamivudine. Clinical pharmacokinetics. 1999 Jan; 36(1):41-66.

15. Lamivudine. https://go.drugbank.com/drugs/DB00709[Cited on 4th November 2021].
16. "List of dosage forms and strength of Lamivudine" https://www.1mg.com/search/all?name=lamivudine [Cited on 12th Nov 2021].
17. Hirtz J. Importance of analytical methods in pharmacokinetic and drug metabolism studies. Biopharmaceutics & drug disposition. 1986 Jul;7(4):315-26.
18. "Bioanalytical Techniques - An Overview" https://www.pharmatutor.org/articles/bioanalytical-techniques-overview[Cited on 26th Nov 2021].
19. Sharma R, Mehta K. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian journal of pharmaceutical sciences. 2010 Jul;72(4):527.
20. Rangapriya M, Rajendran NN, Pharm M. Validation of UV spectroscopy for simultaneous estimation of stavudine, lamivudine and nevirapine in tablet formulations. International Journal of Pharma Sciences and Research. 2014;5(10):665-74.
21. Deepali G, Elvis M. UV Spectrophotometric Method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient in its tablet formulation. Journal of Young Pharmacists. 2010 Oct 1;2(4):417-9.
22. Gupta A, Bhagawati ST, Muddukrishna BS, Jagadish PC, Bhat K. Estimation of stavudine, lamivudine and nevirapine by chemometric UV spectroscopic method. Ind J Pharm Edu Res. 2012 Jul; 46(3):197-205.
23. Grangeiro Jr S, Albuquerque MM, Santana DP, Pimentel MF, Silva RH, Simões SS. Simultaneous spectrophotometric determination of lamivudine and zidovudine in fixed dose combinations using multivariate calibration. Química Nova. 2011; 34:859-63.
24. Bhavsar DS, Patel BN, Patel CN. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharmaceutical methods. 2012 Jul 1; 3(2):73-8.
25. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simeltaneous determination of Lamivudine, tenofovir, and Dolutegravir in bulk and their tablet dosage form. Future Journal of Pharmaceutical Sciences. 2015 Dec 1; 1(2):73-7.
26. Noorbasha K, Nurbhasha S. A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. Future Journal of Pharmaceutical Sciences. 2020 Dec; 6(1):1-10.
27. Singh VD, Daharwal SJ. Optimization of RP-HPLC Method for Simultaneous Estimation of Lamivudine and Raltegravir in Binary Mixture by Using Design of Experiment. Eurasian Journal of Analytical Chemistry. 2017; 12(3):179-95.
28. Tamilarasan S, Malathi S. Analytical Method Development and Validation for the Estimation of Dolutegravir and Lamivudine in Bulk From by RP-HPLC.Journal of Pharmacetical Science and Research. 2021 Apr 1; 13(4):227-31.
29. Mastanamma S, Jyothi JA, Saidulu P, Varalakshmi M. Development and validation of RP-HPLC method for the simultaneous estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir bulk and their combined dosage form. Pharmaceutical Methods. 2018 Dec 20; 9(2):49-55.
30. Gu Y, Zeng B, Sherma J. Development of quantitative HPTLC methods for dolutegravir, lamivudine, and tenofovir disproxil fumarate in a combination pharmaceutical product using a model process published earlier for transfer of minilab TLC screening methods to HPTLC-densitometry. Acta Chromatographica. 2020 Sep; 32(3):199-202.
31. Baig MS, Dehghan MH. Stability-Indicating HPTLC Method Lamivudine and Tenofovir from its tablet dosage form. Journal of medicinal chemistry and drug discovery. 2015 Jan: 463-74.
32. Barsagade AG, Kakde RB. Validated specific HPTLC method for simultaneous estimation of zidovudine and lamivudine in its combined dosage form. World Journal of Pharmaceutical Sciences. 2015 Aug 1:1693-700.
33. Verma S, Mullick P, Bhatt MS, Siddiqui N, Alam O, Bala I, Khan SA. Bioanalytical method development and validation for the simultaneous estimation of lamivudine and stavudine in human plasma by HPLC. Acta Poloniae Pharmaceutica. 2010 Jul 1; 67(4):429-37.
34. Singh AV, Nath LK, Pani NR. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. Journal of pharmaceutical analysis. 2011 Nov 1;1(4):251-57.

35. Marakatham S, Shanmugapandiyan P. Bioanalytical method development and validation of doravirine, lamavudine and tenofovir disoproxil fumarate using HPLC in human plasma. Research Journal of Pharmacy and Technology. 2021 Aug 1; 14(8):4087-91.
36. Alnouti Y, Lewis SR, White CA, Bartlett MG. Simultaneous determination of zidovudine and lamivudine from rat tissues by liquid chromatography/tandem mass spectrometry. Rapid Communication In Mass Spectrometry, 2005; 19: 503–508.
37. Malm M, Römsing S,Obua C,BergqvistY. Determination of Lamivudine, Zidovudine, and Nevirapine in Capillary Blood Sampled on Filter Paper by LC, Journal of Chromatographic Science, November/December 2009; 47,855-862.