

Review article on Analytical Techniques of Lamivudine Determination in Different Matrices

Roshdy E. Saraya¹, Magda Elhenawee², Hanaa Saleh², Mahmoud M. Sebaiy^{3,*}

¹Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Port Said University, Port Said, 42511, Egypt.

²Department of Analytical Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

³Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, 44519, Egypt.

Abstract

In this literature review, we will introduce most of up-to-date reported methods that have been developed for determination of lamivudine in its pure form, combined form with other drugs, combined form with degradation products, and in biological samples.

Corresponding author: Mahmoud M. Sebaiy, Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, 44519, Egypt. E-mail: mmsebaiy@zu.edu.eg; sebaiym@gmail.com Tel: 01062780060. Fax: 0552303266

Keywords: DNA, HIV-1, HIV-2

Received: Dec 13, 2020

Accepted: Dec 31, 2020

Published: Jan 05, 2021

Editor: Fatma Mohammed Mady, Department of Pharmaceutics, Minia University, Egypt.

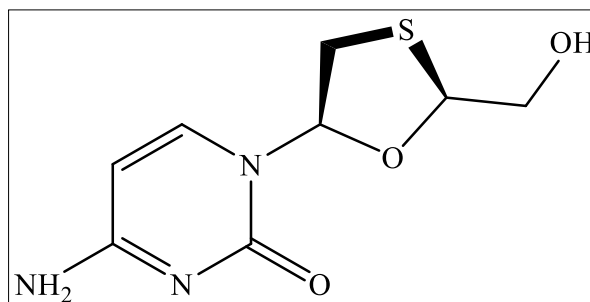
Introduction

Lamivudine

(LAM) is an analogue of cytidine. It can inhibit both types HIV-1 and HIV-2 reverse transcriptase and also the reverse transcriptase of hepatitis B virus. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis [1]. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated [2].

As such, in this literature review, we will introduce most of up-to-date reported methods that have been developed for determination of LAM in its

pure form, combined form with other drugs, combined form with degradation products, and in biological samples.



Review of Analytical Methods

Various techniques were used for the analysis of RIT in pure forms, in their pharmaceutical formulations and in biological fluids. The available reported methods in the literature can be summarized as follows: Tab 1.

Table 1. Spectrophotometric Methods

Drugs	Method or reagent	λ_{\max}	Ref
LAM and zidovudine	First derivative spectrophotometry	239.5 and 245.3 nm for LAM and 225.1 and 251.5 nm for zidovudine	[3]
LAM and stavudine	First derivative spectrophotometry	280 for stavudine and 300 nm for LAM.	[4]
LAM and stavudine	3-methyl-2-benzothiazolinone hydrazone hydrochloride and ferric chloride	660 nm for LAM and 630 nm for stavudine	[5]
LAM	chloramine-T and methyl orange, or chloramine-T and indigo carmine	520nm or 610nm	[6]
LAM and zidovudine	derivative spectrophotometry	246 nm for LAM and 263 nm for zidovudine	[7]
LAM	N HCl 0.1 N NaOH	279.6 nm 269.8 nm	[8]
Tenofovir, Disoproxil and LAM	Simultaneous equation method Multicomponent analysis Derivative spectroscopy method	247, 259 and 272 nm	[9]
LAM and zidovudine	Derivative Spectrophotometry	242, and 236 nm	[10]
LAM	methyl orange and indigocarmine	520n and 610 nm	[11]
LAM and zidovudine	UV spectroscopy and multivariate calibration	250 and 267 nm	[12]
LAM and efavirenz	overlain spectra method	271 and 247 nm	[13]
LAM	chloranilic acid and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone	221 and 230 nm	[14]
LAM, nevirapine and zidovudine	overlain spectra method	280.2nm, 312nm and 266.8nm	[15]
LAM, Sofosbuvir, and Ritonavir	Silver nanoparticles synthesis	421 nm for Sofosbuvir and Ritonavir and at 425 nm for Lamivudine LAM	[16]

2. Chromatographic Methods

Table 2 a. HPLC

Matrix	Column	Mobile phase	system	Ref
plasma	Aquasil C ₁₈ column	ACN : water (15:85 v/v)	HPLC–MS/MS	[17]
Tablet	Spherisorb [®] C ₁₈ analytical column	methanol: water: ACN (70:20:10 (v/v/v))	HPLC-UV 265 nm	[3]
plasma	a Shim-pack [®] C ₈ column	Sodium dihydrogen phosphate monohydrate (10 mM): methanol: ACN (94:3:3, v/v/v, pH 4.8)	HPLC-UV 270 nm	[18]
plasma	Phenomenex C ₈ column	A gradient elution with 20 mM ammonium acetate buffer with pH 4.5 : ACN	HPLC–MS/MS	[19]
Tablet	Symmetry C ₁₈ column	methanol: water (20:80 v/v)	HPLC-UV 270 nm	[4]
Tablet	C ₁₈ column	A gradient elution with 80% of 10 mM acetate buffer (pH 3.5): 20% methanol: 50% CAN: 50% isopropyl alcohol.	HPLC-UV 270 nm	[20]
Plasma and saliva	Zorbax SB-C ₁₈ column	0.005 M di potassium hydrogen phosphate solution in water (pH 6.8): methanol (92:8 v/v).	HPLC-UV 270 nm	[21]
Tablet	HiQ Sil C ₁₈ column	0.01 M potassium di hydrogen orthophosphate (pH 3.0): methanol (55:45 v/v)	HPLC-UV 272 nm	[22]
plasma	C ₁₈ column	a mixture of phosphate buffer (0.05 M) containing TEA (1 mL/L pH 3.5): methanol (91:9, v/v)	HPLC-UV 276 nm	[23]
plasma	Lichrospher [®] RP- C ₁₈ column	20 mM ammonium acetate: methanol containing 1% of acetic acid (60:40 v/v)	HPLC–MS/MS	[24]
plasma	a phenyl column with Phenomenex C ₁₈ guard column	5% methanol in 20 mM dibasic phosphate buffer (pH 6).	HPLC-UV 256 nm	[25]
plasma	octylsilane column	20 mM sodium phosphate buffer with (8 mM 1 octane sulfonic acid sodium salt): ACN (86:14, v/v)	HPLC-UV 265 nm	[26]
plasma	C ₁₈ analytical column	CAN: water (9:91, v/v)	HPLC-UV 271 nm	[27]
plasma	Aquasil C ₁₈ column	ACN: water (15:85, v/v)	HPLC–MS/MS	[28]
plasma	A Symmetry Shield RP C ₁₈ column	A gradient elution with acetate buffer (20 mM potassium acetate pH 4.60): ACN	HPLC-UV 260 nm	[29]
plasma	a Shiseido C ₈ column	a gradient elution with methanol: water (80:20, v/v) and water, both containing 10 mM ammonium acetate	HPLC –MS/MS	[30]
Plasma	a Spherisorb [®] C ₁₈ analytical column	methanol: water (75 : 25, v/v)	HPLC-UV at 265 nm	[31]
Plasma	a C ₁₈ column	CAN: water (13:87, v/v)	HPLC-UV at 220 nm	[32]

Tablet	Thermo Hypersil Gold C ₁₈ column	A gradient elution with 20 mM sodium phosphate buffer (pH 3.5) with phosphoric acid : methanol	HPLC-UV at 265 nm	[33]
Plasma	a C ₁₈ column	0.01M sodium dihydrogen phosphate : methanol: ACN (4 : 2 : 3 v/v/v)	HPLC-UV at 285 nm	[7]
Plasma	Phenyl column C ₁₈ column	ACN: 0.085% phosphoric acid (12:88, v/v)	HPLC-UV at 270 nm	[34]
Plasma	Aquasil® C ₁₈	A gradient elution with 0.05% FA in either water or methanol	HPLC – MS/MS	[35]
Plasma	Zorbax® C ₁₈ column	methanol: water: phosphate buffer (pH 5.65) (80:10:10; v/v/v)	HPLC-UV at 275 nm	[36]
Plasma	A Phenomenex C18	Methanol: Water (85:15%v/v)	HPLC-UV at 270 nm	[37]
Plasma and tissues	a phenyl column	8% ACN in 5 mM 1-heptane sulfonic acid dissolved in 30 mM AF buffer (pH 3.3).	HPLC-UV at 254 nm	[38]
Plasma	a Vydac C ₁₈ column	A gradient elution, both CAN and ultrapure water solvents contained 0.2% FA.	HPLC – MS/MS	[39]
Rabbit plasma	Hypersil BDS C-18 column	0.25% Triethylamine buffer (pH 3.0): CAN (70:30, v/v)	HPLC-UV at 256 nm	[40]
Plasma	Zorbax SB C ₈ column	A gradient elution with methanol: acetic acid sodium acetate buffer (pH 3.9)	HPLC-UV at 260 nm	[41]
Plasma	Prontosil C ₁₈ column	1 mM ammonium acetate in water (pH 6.5 ± 0.3): ACN (50:50 v/v)	HPLC – MS/MS	[42]
Plasma	A Chromolith C ₁₈ column	50 mM sodium dihydrogen phosphate : TEA (996:4 v/v)	HPLC-UV at 278 nm	[43]
Plasma	An ACE 5 CN column	0.5% FA in water: ACN (55:45, v/v)	HPLC – MS/MS	[44]
Rat tissue	a C ₁₈ column	methanol: 7.5 mM ammonium acetate (30:70, v/v)	HPLC – MS/MS	[45]
Plasma	Pack VP - ODS C ₁₈ column	phosphate buffer (pH 7.0): ACN : methanol (91:0.1:9)	HPLC-UV at 274 nm	[46]
Plasma	a C ₁₈ column	A gradient with 0.1% FA in water and 0.1% FA in methanol	HPLC – MS/MS	[47]
Tablet	a C ₁₈ column	water: methanol (60:40 v/v)	HPLC-UV 270 nm	[48]
Plasma	A Phenomenex C ₁₈ column	A aqueous solution of 15% ACN and 0.1% acetic acid	HPLC – MS/MS	[49]
Tablet	A bondapak C ₁₈	0.02 M tri-sodium citrate and methanol (70:30 v/v)	HPLC-UV at 266 nm	[50]
Tablet	A thermo BDS C ₁₈ column	A formic acid and methanol in the ratio of 50:50	HPLC-UV at 264 nm	[51]
Plasma	a C ₈ column	A gradient elution with 10 mM potassium phosphate, 3% ACN, and methanol	HPLC-UV at 272 nm	[52]
Tablet	a Hypersil BDS, C ₁₈ column	o- phosphoric acid: methanol (70:30)	HPLC-UV at 220 nm	[53]
Plasma	A Peerless Basic C ₁₈ column	0.1% formic acid in water: methanol (15:85, v/v)	HPLC – MS/MS	[54]
Tablet	a C ₁₈ column	methanol and water (89:11 v/v)	HPLC-UV at 272 nm	[55]
Tablet	a Diamonsil C ₁₈ column	0.025 mol ammonium acetate (pH 3.9 ± 0.1)-methanol (90:10).	HPLC-UV at 270 nm	[56]

Plasma	a C ₁₈ column	A gradient elution with 10 mM acetate buffer (pH 6.5)- ACN	HPLC-UV at 265 nm	[57]
Plasma	a Hypurity Advance C ₁₈	ACN :0.1% FA (76:24, v/v)	HPLC – MS/MS	[58]
Tablet	A LunaC ₁₈	A gradient elution with 50mM ammonium acetate buffer (pH = 6.8) and methanol	HPLC-UV at 265 nm	[59]
plasma	a Hypersil BDS, C18 column	0.1 M ammonium acetate buffer in 0.5% acetic acid, v/v and methanol (40:60, v/v)	HPLC-UV at 270 nm	[60]
Tablet	An YMC pack C ₈ column	buffer pH 3.5: methanol (90:10 v/v)	HPLC-UV at 265 nm	[61]
Tablet	a Kromasil C ₁₈ analytical column	methanol: 10 mM phosphate buffer (pH 5.0) (70:30 v/v).	HPLC-UV at 254 nm	[62]
Tablet	A Luna C ₁₈	0.1 % triethylamine (pH 5.11: ACN (70:30)	HPLC-UV at 245 nm	[63]
Tablet	a Luna hydrophilic interaction column	ACN /10 mM ammonium formate (95:5, v/v)	HPLC – MS/MS	[64]
Tablet	a C ₁₈ column	A gradient elution with 0.05 M Phosphate buffer (pH 6.2): ACN	HPLC-UV 260 nm	[65]
Tablet	A Phenomenex Luna C ₁₈ column	ACN : methanol: water 30: 45: 25 (v/v/v)	HPLC-UV 258 nm	[66]

Table 2 b. HPTLC

Matrix	Stationary phase	Mobile phase	detector	Ref
Tablet	silica-gel 60 F ₂₅₄ plate	toluene/chloroform/methanol (1:6:3 v/v/v)	UV- 276 and 271 nm	[67]
Tablet	silica-gel 60 F ₂₅₄ plate	Acetone: chloroform: methanol (4: 4: 2 v/v/v)	UV- 265nm	[68]
Tablet	silica-gel 60 F ₂₅₄ plate	chloroform: methanol: toluene (8: 2: 2, v/v/v)	UV- 265nm	[69]
Tablet	silica-gel 60 F ₂₅₄ plate	ethyl acetate, methanol, toluene and conc ammonia (38.7:19.4:38.7:3.2, v:v:v:v)	UV- 254nm	[70]
Tablet	silica-gel 60 F ₂₅₄ plate	n-hexane: chloroform: methanol (1:7:2 v/v/v)	UV- 275 nm	[71]

Other Methods

Titrimetry [6, 11], capillary electrophoresis [72, 73], chemometry [74], Voltametry [75].

Conclusion

This literature review represents an up to date survey about all reported methods that have been developed for determination of the anticancer drug, lamivudine in its pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, electrochemistry, etc...

References

1. John Betts and Sue Ho, *'Martindale': from abrus to zotarolimus—130 years of pharmacy knowledge*. Dementia, 2016. 14(53): p. 583m.
2. Ching Lung Lai and Man Fung Yuen, *Profound suppression of hepatitis B virus replication with lamivudine*. Journal of medical virology, 2000. 61 (3): p. 367-373.
3. B.Uslu and S.A.Özkan, *Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography-UV methods*. Analytica Chimica Acta, 2002. 466(1): p. 175-185.
4. Namita Kapoor, Sateesh Khandavilli, and Ramesh Panchagnula, *Simultaneous determination of lamivudine and stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography*. Journal of pharmaceutical and biomedical analysis, 2006. 41(3): p. 761-765.
5. DG Sankar, et al., *Spectrophotometric determination of lamivudine and stavudine*. Indian journal of pharmaceutical sciences, 2002. 64(5): p. 504.
6. K Basavaiah and BC Somashekar, *Titrimetric and spectrophotometric determination of lamivudine in pharmaceuticals*. Indian Journal of Chemical Technology, 2006. 13(1): p. 7-12.
7. Vorname Nachname, *Derivative-differential UV spectrophotometry and compensation technique for the simultaneous determination of zidovudine and lamivudine in human serum*. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2004. 59(2): p. 106-111.
8. S Shalini, et al., *Application of uv-spectrophotometric methods for estimation of lamivudine in tablets*. Digest Journal of Nanomaterials & Biostructures (DJNB), 2009. 4(2): p. 357-360.
9. R Sharma and K Mehta, *Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz*. Indian journal of pharmaceutical sciences, 2010. 72 (4): p. 527-530.
10. Mahmoud Reza Sohrabi and Mahshid Tayefeh Zarkesh, *Spectra resolution for simultaneous spectrophotometric determination of lamivudine and zidovudine components in pharmaceutical formulation of human immunodeficiency virus drug based on using continuous wavelet transform and derivative transform techniques*. Talanta, 2014. 122 (1): p. 223-228.
11. K Basavaiah, BC Somashekar, and V Ramakrishna, *Rapid titrimetric and spectrophotometric assay methods for the determination of lamivudine in pharmaceuticals using iodate and two dyes*. Journal of Analytical Chemistry, 2007. 62(6): p. 542-548.
12. Severino Grangeiro Jr, et al., *Simultaneous spectrophotometric determination of lamivudine and zidovudine in fixed dose combinations using multivariate calibration*. Química Nova, 2011. 34(5): p. 859-863.
13. A Manikanta Kumar, et al., *Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical dosage form*. Journal of Advanced Pharmacy Education & Research Oct-Dec, 2012. 2(4): p. 210-214.
14. Madu, K.C., P. Ukoha, and A. Attama, *Spectrophotometric determination of lamivudine using chloranilic acid and 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ)*. American Journal of Analytical Chemistry, 2011. 2(07): p. 849.

15. Vaishali P Nagulwar and Kishor P Bhusari, *A validated UV spectrophotometric method for the simultaneous estimation of Lamivudine, Nevirapine and zidovudine in combined tablet dosage form.* Journal of Pharmacy Research, 2009. 2(4): p. 666-669.
16. Saraya, R.E., M. Elhenawee, and H. Saleh, *Silver Nanoparticles Synthesis for Sensitive Spectrophotometric Determination of Sofosbuvir, Lamivudine, and Ritonavir in Pure Forms and Pharmaceutical Dosage Forms.* Journal of AOAC International, 2020. 103(1): p. 140-147.
17. A.S.Pereira, et al., *Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry.* Journal of Chromatography B: Biomedical Sciences and Applications, 2000. 742(1): p. 173-183.
18. Kano, E.K., et al., *Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies.* International journal of pharmaceutics, 2005. 297(1-2): p. 73-79.
19. Fan, B., M.G. Bartlett, and J.T. Stewart, *Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch.* Biomedical Chromatography, 2002. 16(6): p. 383-389.
20. N.Kapoor, S.Khandavilli, and R.Panchagnula, *Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography.* Analytica Chimica Acta, 2006. 570 (1): p. 41-45.
21. Hoetelmans, R.M., et al., *Quantitative determination of (-)-2'-deoxy-3'-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection.* Journal of Chromatography B: Biomedical Sciences and Applications, 1998. 713(2): p. 387-394.
22. Kumar, D.A., G.S. Rao, and J. Rao, *Simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage forms by RP HPLC method.* Journal of Chemistry, 2010. 7(1): p. 180-184.
23. G. Bahrami, et al., *High-performance liquid chromatographic determination of lamivudine in human serum using liquid-liquid extraction; application to pharmacokinetic studies.* Journal of chromatography B, 2005. 823(2): p. 213-217.
24. Rita de Cassia E Estrela, Myriam C Salvadori, and Guilherme Suarez-Kurtz, *A rapid and sensitive method for simultaneous determination of lamivudine and zidovudine in human serum by on-line solid-phase extraction coupled to liquid chromatography/tandem mass spectrometry detection.* Rapid communications in mass spectrometry, 2004. 18(10): p. 1147-1155.
25. Yazen Alnouti, Catherine A White, and Michael G Bartlett, *Determination of lamivudine in plasma, amniotic fluid, and rat tissues by liquid chromatography.* Journal of Chromatography B, 2004. 803(2): p. 279-284.
26. Bin Fan and James T Stewart, *Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC.* Journal of pharmaceutical and biomedical analysis, 2002. 28(5): p. 903-908.
27. Alicia Tarinas, et al., *Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma.* Farmacia Hospitalaria, 2007. 31(4): p. 243-247.
28. Kathryn B Kenney, et al., *Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry.* Journal of pharmaceutical and biomedical analysis, 2000. 22(6): p. 967-983.
29. Verweij-van CPWGM Wissen, RE Aarnoutse, and DM Burger, *Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography.* Journal of chromatography B, 2005. 816(1-2): p. 121-129.
30. Zhou Li, et al., *Simultaneous determination of lamivudine, stavudine and nevirapine in human*

- plasma by LC-MS/MS and its application to pharmacokinetic study in clinic.* Biomedical Chromatography, 2010. 24(9): p. 926-934.
31. Sibel A Ozkan and Bengi Uslu, *Rapid HPLC assay for lamivudine in pharmaceuticals and human serum.* Journal of liquid chromatography & related technologies, 2002. 25(9): p. 1447-1456.
 32. Liu, X., et al., *Development and validation of LC coupled with SPE for simultaneous determination of lamivudine, oxymatrine and its active metabolite matrine in dog plasma.* Chromatographia, 2006. 63 (9-10): p. 483-486.
 33. Weerasak Samee, et al., *Simultaneous determination of Lamivudine, Stavudine and Nevirapine in the presence of their acid-induced degradation products by HPLC.* Thai Pharm Health Sci J, 2007. 2(1): p. 39-45.
 34. Joanna J Zheng, Steven T Wu, and Thomas A Emm, *High-performance liquid chromatographic assay for the determination of 2'-deoxy-3'-thiacytidine (lamivudine) in human plasma.* Journal of Chromatography B: Biomedical Sciences and Applications, 2001. 761(2): p. 195-201.
 35. JByung Hwaung, et al., *Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry.* Biomedical Chromatography, 2007. 21(10): p. 1095-1104.
 36. A Savaşer, et al., *Determination of abacavir, lamivudine and zidovudine in pharmaceutical tablets, human serum and in drug dissolution studies by HPLC.* Chromatographia, 2007. 65(5-6): p. 259-265.
 37. S Jayaseelan, et al., *Bioanalytical method development and validation of Lamivudine by RP-HPLC method.* Int J Chem Tech Res, 2010. 2(1): p. 163-7.
 38. Yazen Alnouti, Catherine A White, and Michael G Bartlett, *Simultaneous determination of zidovudine and lamivudine from rat plasma, amniotic fluid and tissues by HPLC.* Biomedical Chromatography, 2004. 18(9): p. 641-647.
 39. Stefania Notari, et al., *Simultaneous determination of lamivudine, lopinavir, ritonavir, and zidovudine concentration in plasma of HIV-infected patients by HPLC-MS/MS.* IUBMB life, 2012. 64(5): p. 443-449.
 40. 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): p. 251-257.
 41. Mikaela Malm, et al., *Determination of lamivudine, zidovudine, and nevirapine in capillary blood sampled on filter paper by LC.* Journal of Chromatographic Science, 2009. 47(10): p. 855-862.
 42. Rajani Kumar Valluru, Praveen Kumar, and Naveen Babu Kilaru, *High throughput LC-MS/MS method for simultaneous determination of tenofovir, lamivudine and nevirapine in human plasma.* Journal of Chromatography B, 2013. 931(1): p. 117-126.
 43. Mahmoud Alebouyeh and Hossein Amini, *Rapid determination of lamivudine in human plasma by high-performance liquid chromatography.* Journal of Chromatography B, 2015. 975(1): p. 40-44.
 44. Manish Yadav, et al., *Selective determination of antiretroviral agents tenofovir, emtricitabine, and lamivudine in human plasma by a LC-MS-MS method for a bioequivalence study in healthy Indian subjects.* Journal of chromatographic science, 2010. 48(9): p. 704-713.
 45. Yazen Alnouti, et al., *Simultaneous determination of zidovudine and lamivudine from rat tissues by liquid chromatography/tandem mass spectrometry.* Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 2005. 19(4): p. 503-508.
 46. Li-hua Shao, et al., *Determination of Lamivudine Concentration in Human Serum by HPLC.* Chinese Journal of Pharmaceutical Analysis, 2002. 22(1): p. 53-54.
 47. W Kromdijk, et al., *Development and validation of an assay for the simultaneous determination of zidovudine, abacavir, emtricitabine, lamivudine, tenofovir and ribavirin in human plasma using liquid*

- chromatography–tandem mass spectrometry*. Journal of Chromatography B, 2013. 919(1): p. 43-51.
48. Predrag Djurdjevic, et al., *Chemometric Optimization of a RP-HPLC Method for the Simultaneous Analysis of Abacavir, Lamivudine, and Zidovudine in Tablets*. Analytical letters, 2004. 37 (13): p. 2649-2667.
49. Joseph E Rower, et al., *Validation of a sensitive LC/MS/MS method for the determination of zidovudine and lamivudine in human plasma*. Biomedical Chromatography, 2012. 26(1): p. 12-20.
50. KK Nerurkar, et al., *Concurrent assay of lamivudine and zidovudine from combination tablets*. Indian journal of pharmaceutical sciences, 2003. 65(4): p. 412- 417.
51. SK Patro, et al., *Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation*. Journal of Chemistry, 2010. 7(1): p. 117-122.
52. acqueline De Souza, et al., *LC–UV methodology for simultaneous determination of lamivudine and zidovudine in plasma by liquid–liquid extraction*. Chromatographia, 2009. 69(2): p. 231-235.
53. MS Palled, et al., *Reverse Phase High Performance Liquid Chromatographic Determination Of Zidovudine And Lamivudine In Tablet Dosage Form*. Indian journal of pharmaceutical sciences, 2005. 67 (1): p. 110-117.
54. Valluru Rajani Kumar, et al., *High throughput LC–MS/MS method for simultaneous determination of zidovudine, lamivudine and nevirapine in human plasma*. Journal of Chromatography B, 2013. 921 (1): p. 9-14.
55. DK Mandloi, et al., *Method development and validation of RP-HPLC in the application of in vitro dissolution study of lamivudine in bulk drug and tablet formulation*. J Chem Pharm Res, 2009. 1(1): p. 286-296.
56. Meng LI, Jing LU, and Xin DI, *RP-HPLC determination of lamivudine and its related substances*. Chinese Journal of Pharmaceutical Analysis, 2009. 29(7): p. 1216-1219.
57. Triporn Wattananat and Wiyada Akarawut, *Simultaneous determination of stavudine and lamivudine in human plasma by high performance liquid chromatography and its application to a bioavailability study*. Southeast Asian Journal of Tropical Medicine and Public Health, 2010. 41(2): p. 369- 377.
58. Murali Krishna Matta, et al., *Simultaneous quantitation of lamivudine, zidovudine and nevirapine in human plasma by liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study*. Acta Pharmaceutica Sinica B, 2012. 2(5): p. 472-480.
59. Moji Christianah Adeyeye and Anjali Joshi, *Reversed Phase LC-UV method development and validation for simultaneous determination of three antiretrovirals: lamivudine, zidovudine, nevirapine and possible degradants in a fixed dose pharmaceutical product*. Journal of Pharmaceutical Technology and Drug Research, 2012. 1(1): p. 1-4.
60. Utpal Nandi, et al., *Development and validation of an HPLC-UV method for simultaneous determination of zidovudine, lamivudine, and nevirapine in human plasma and its application to pharmacokinetic study in human volunteers*. Drug testing and analysis, 2013. 5(6): p. 485-491.
61. C Balasekarreddy, et al., *Validated HPLC Method for determination of Lamivudine and Stavudine in their formulations*. Pharmanest, 2010. 1(1): p. 22-8.
62. Dhara S Bhavsar, Bhavini N Patel, and Chhaganbhai N Patel, *RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form*. Pharmaceutical methods, 2012. 3(2): p. 73-78.
63. A Manikanta Kumar, et al., *Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical Dosage Form*. Journal of Advanced Pharmacy Education & Research Oct-Dec, 2012. 2 (4): p. 232-238.

64. Hye Young Ji, et al., *Quantification of lamivudine in human plasma by hydrophilic interaction chromatography-tandem mass spectrometry*. Journal of separation science, 2010. 33(6-7): p. 948-954.
65. Nagasarapu Mallikarjuna Rao and Dannana Gowri Sankar, *Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form*. Future Journal of Pharmaceutical Sciences, 2015. 1 (2): p. 73-77.
66. K Anandakumar, et al., *RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation*. Journal of analytical chemistry, 2013. 68(9): p. 815-821.
67. Habte, G., A. Hymete, and A.-M.I. Mohamed, *Simultaneous separation and determination of lamivudine and zidovudine in pharmaceutical formulations using the HPTLC method*. Analytical Letters, 2009. 42(11): p. 1552-1570.
68. T Sudha, VR Ravikumar, and PV Hemalatha, *Validated HPTLC method for simultaneous determination of lamivudine and abacavir sulphate in tablet dosage form*. International Journal of Pharmaceutical Sciences and Research, 2010. 1(11): p. 107.
69. P Chandra, et al., *Application of high-performance thin-layer chromatographic method for the simultaneous determination of Lamivudine and tenofovir disoproxil fumarate in pharmaceutical dosage form*. Journal of the Chilean Chemical Society, 2011. 56(2): p. 702-705.
70. DH Shewiyo, et al., *Development and validation of a normal-phase HPTLC method for the simultaneous analysis of lamivudine, stavudine and nevirapine in fixed-dose combination tablets*. Journal of pharmaceutical and biomedical analysis, 2011. 54 (3): p. 445-450.
71. Gebremedhin Solomon, et al., *HPTLC-densitometric method development and validation for simultaneous determination of lamivudine, nevirapine and zidovudine in fixed dose combinations*. Thai Journal of Pharmaceutical Sciences, 2011. 35(2): p. 77-88.
72. Bin Fan and James T Stewart, *Determination of lamivudine/didanosine/saquinavir in human serum using capillary zone electrophoresis*. Journal of liquid chromatography & related technologies, 2002. 25 (2): p. 241-249.
73. R Sekar and S Azhaguvel, *Simultaneous determination of HIV-protease inhibitors lamivudine and zidovudine in pharmaceutical formulations by micellar electrokinetic chromatography*. Journal of pharmaceutical and biomedical analysis, 2005. 39(3-4): p. 653-660.
74. Abd El-Maaboud I Mohamed and Workalemahu Mikre, *Determination of lamivudine and stavudine in pharmaceutical preparations using chemometrics-assisted spectrophotometry*. Saudi Pharmaceutical Journal, 2009. 17(4): p. 275-281.
75. Percio Farias, M.A., Arnaldo A Castro, and Ana Isa P Cordoves, *Ultrace determination of the antiretroviral drug lamivudine in diluted alkaline electrolyte by adsorptive stripping voltammetry*. ECS Transactions, 2012. 43(1): p. 267-287.