MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Review on Analytical Methods for Determination of Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz in Different Matrices

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Abstract

Acquired Immunodeficiency Syndrome (AIDS) is a dangerous and fatal disease. Persons with AIDS can lead long, healthy lives if they receive early diagnosis, appropriate medical care, and medication. The combination of HIV medications is known as antiretroviral therapy (ART) is used to reduce transmission and stop the development of AIDS.This article is made for presenting a mini-review of different analytical methods for determination of some antiviral drugs namely lamivudine (LAM), tenofovir disoproxil fumarate (TDF) and efavirenz (EFA) either in pharmacopeia or not, in order to provide other researchers with up-to-date knowledge of the analytical techniques applied to determine the previously mentioned drugs either alone or combined with other antiretroviral agents. This article also includes essential data about the cited drugs, involving physical and chemical characteristics, as well as its pharmacological actions. The collected methods in this mini review include pharmacopeia, chromatographic (HPLC, TLC and UPLC), spectrophotometric and electrochemical methods. All methods are validated according to ICH.

Keywords: Analytical methods, Efavirenz, Lamivudine, Tenofovir Disoproxil Fumarate.

1. Introduction

Acquired Immunodeficiency Virus (AIDS) is considered the last stage of Human immunodeficiency virus (HIV) infection (Bhaskar et al., 2021). As soon as people infected by HIV, they may get long term symptoms such as: fatigue, fever, diarrhea, weight loss and swollen lymph glands (Miedzinski, 1992). Fixed dose combination of lamivudine, tenofovir disoproxil fumarate and efavirenz is one of the best antiretroviral drugs for treatment of AIDS. This combination was approved by FDA as symfi and symfi lo tablets in 2018 (Medicines, 2024). LAM ("Drug bank online, Lamivudin," 2024), with a chemical name of 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one (Figure 1A), is a nucleoside reverse transcriptase inhibitor (NRTI) that is used against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV) by preventing the HIV enzyme reverse transcriptase and B virus polymerase from their functioning. Lam is a white or off white crystalline powder that is sparingly soluble in water with a molecular

weight of (229.26 g/mol). TDF ("Drug bank online, Tenofovir disoproxil fumarate," n.d.), with a chemical name of [[(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethyl-(propan-) phosphoryl] oxymethyl propan-2-yl carbonate; (E)-but-2-enedioic acid (Figure 1B), is a reverse transcriptase inhibitor that is used to treat AIDS by preventing HIV from replication. TDF is white and fine powder and slightly soluble in water and ethanol with a molecular weight 635.5 g/mol. EFA("Drug bank online, Efavirenz," n.d.), with a chemical name of (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one (Figure 1C), is a non-nucleoside reverse transcriptase inhibitors (NNRTIs) that is used to treat human immunodeficiency virus (HIV) with other medications by decreasing the risk of spreading and replication of the (HIV). EFA is white to slightly pink crystalline solid and practically insoluble in water with 315.67 g/mol.

2. Analytical methods

2.1. Official analytical methods for the analysis of LAM, TDF and EFV.

LAM is determined in British Pharmacopeia (BP)(Commission and British Pharmacopoeia, 2020) and United States Pharmacopeia (USP) (Naproxen et al., 2020) by liquid chromatography with methanol and ammonium acetate (5:95) as a mobile phase and detection at 277 nm while, there is no official analytical method for determination of TDF.

EFA can be analyzed in United States Pharmacopeia (USP), by liquid chromatography with Acetonitrile, trifuoroacetic acid, and water (55:0.05:45) as a mobile phase and detection at 250 nm(Spectroscopy and Spectroscopy, 2020) and there is no determination of EFA in British Pharmacopeia (BP).

2.2 Reported analytical methods for the analysis of LAM, TDF and EFV.

2.2.1. Chromatographic methods

2.2.1.1.High-performance liquid chromatography

Different high performance liquid chromatographic (HPLC) methods were reported for determination of LAM, TDF and EFA in different matrices using various detectors as presented in **(Table.1)**

2.2.1.2. Ultra-Performance Liquid chromatographic methods

A validated RP-UPLC method was reported for determination of LAM and its impurities by using acquit UPLC BEH Phenyl C18 column 2.1 mm × 100 mm, 1.7 µm.(Reddy Yellampalli et al., 2018)

2.2.1.3.Thin Liquid chromatographic methods

High-performance thin-layer liquid chromatography (HPTLC) was reported for separation of EFA with LAM and TDF by using Merck HPTLC aluminum plates pre-coated with silica gel G60 F_{254} as stationary phase and chloroform–methanol–toluene (9:1.2:0.3, v/v) as mobile phase (More et al., 2013)

2.2.2. Spectroscopic methods

UV Spectroscopic method was reported for determination of LAM in tablet dosage form by using analytical grade water as a diluent.(Sonar et al., 2017)

UV Spectroscopic method was used for determination of TDF after complexation with ammonium molybdate-stannous Chloride and picric acid (Onah and Ajima, 2011). UV spectrophotometry method can be measured TDF in tablets in the range 200 – 400 nm and the absorption maxima was showed at nm (Rani et al., 2012). UV spectrophotometric methods were applied to separate EFA from LAM and TDF at 247, 272 and 259 nm, respectively by using methanol: water (50:50) as diluent.(Sharma and Mehta, 2010)

Simultaneous estimation of EFA, TDF and LAM in pure and tablet dosage form by UV spectrophotometric methods. (Murugan et al., 2017)

2.2.3. Electrochemical methods

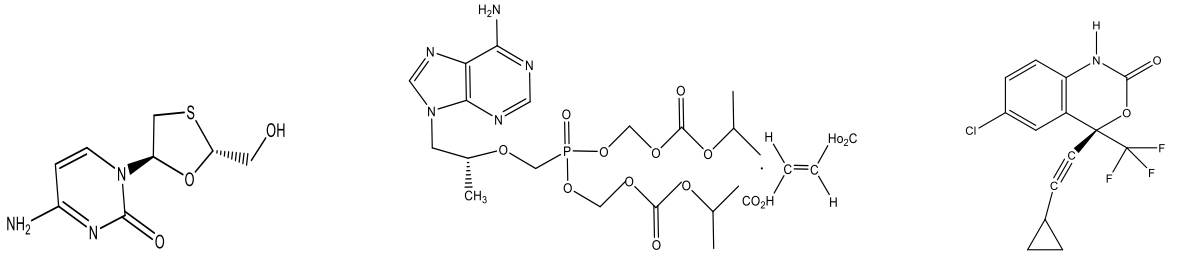
LAM was determined in serum and pharmaceutical dosage form by using hanging mercury drop electrode (HMDE)(Dogan et al., 2005)

TDF was determined by using a composite modified glassy carbon electrode for zirconium oxide-chitosan-multi walled carbon nanotubes (ZrO2-CS-MWCNTs/GCE).(Xiao et al., 2022)

NiO–ZrO2 nanocomposite modified electrode was used as an electrochemical sensor for the determination of EFA (Thapliyal et al., 2015).

Table 1. Summary of HPLC methods published for determination of LAM, TDF and EFA.

Stationary phase	Mobile phase	Detection	Application	Ref.
Shim-pack® CLC-C8(M) column (150 mm x 4.6 mm i.d., particle size 5µm)	sodium dihydrogen phosphate monohydrate, methanol and acetonitrile (94:3:3, v/v/v)	270 nm	LAM in human plasma	(Kano et al., 2005)
Monolithic column (100 x 4.6mm I.D.)	acetonitrile/water (65:35, v/v)	285nm	LAM	(Aboul-Enein and Hefnawy, 2003)
Hypersil BDS C-18 column (250 mm x 4.6 mm, 5 mm)	0.25% Triethylamine buffer (pH 3.0): acetonitrile (70:30, v/v)	256 nm	LAM in rabbit plasma	(Vikram et al., 2011)
CLC C18 (5 m, 25 cm x 4.6 mm i.d.)	Acetonitrile/water mixture (75:25)	259 nm	TDF in spiked human plasma samples and pharmaceutical formulations.	(Taylor et al., n.d.)
C_{18} column	Na HPO buffer, tetra butyl ammonium hydrogen sulfate and acetonitrile	259 nm	TDF in plasma	(Sentenac and Fernandez, 2003)
An atlantis®-dC-18 analytical column	ammonium acetate/methanol (98.5:1.5, v/v)	260 nm	TDF in human plasma	(Barkil et al., 2007)
Waters X-Terra Shield, RP18 50 x 4.6 mm, 3.5 µm column.	Phosphate buffer pH 3.5 and Acetonitrile.	260nm	EFA in plasma	(Gupta et al., 2017)
Kromasil C18 analytical column	Methanol and 10 mM phosphate buffer (70:30)	254 nm	EFA, LAM and TDF	(Bhavsar et al., 2012)
Hypersil C18	Water and acetonitrile (30:70) with phosphate buffer	PDA detection at 260nm	EFA, LAM and TDF	(B. and A., 2014)
Zorbax eclipse XDB-Phenyl column	methanol: buffer (0.1% v/v formic acid in water)(73:27 v/v)	260nm	EFA, LAM and TDF	(Godela et al., 2021)



A B C

Figure 1. Chemical structure of (A) LAM, (B) TDF and (C) EFA.

3. Conclusion

Clear and specific summary of analytical methods for determining LAM, TDF and EFA alone or in combination. The HPLC technique was clearly the most advanced approach, followed by spectroscopy.

4. References

Aboul-Enein, H.Y., Hefnawy, M.M., 2003. High throughput analysis of lamivudine in pharmaceutical preparations using monolithic silica HPLC column. Anal. Lett. 36, 2527–2538. https://doi.org/10.1081/AL-120024340

B., P., A., D., 2014. Analytical method development and validation of invitro dissolution studies of efavirenz, lamivudine and tenofovir disoproxil fumarate in pharmaceutical dosage form by RP-HPLC. J. Glob. Trends Pharm. Sci. 5, 1634–1640.

Barkil, M. El, Gagnieu, M.C., Guitton, J., 2007. Relevance of a combined UV and single mass spectrometry detection for the determination of tenofovir in human plasma by HPLC in therapeutic drug monitoring. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 854, 192–197. https://doi.org/10.1016/j.jchromb.2007.04.015

Bhaskar, D.J., Metry, D.A., B, D.S., 2021. Hematological profile of people living with HIV/AIDS. Int. J. Adv. Res. Med. 3, 507–510. https://doi.org/10.22271/27069567.2021.v3.i2h.298

Bhavsar, D.S., Patel, B.N., Patel, C.N., 2012. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharm. Methods 3, 73–78. https://doi.org/10.4103/2229-4708.103876

Commission, B.P., British Pharmacopoeia, 2020. British Pharmacopoeia 2020 (BP 2020). Br. Pharmacopoeia 2020 II, 640–1074.

Dogan, B., Uslu, B., Suzen, S., Ozkan, S.A., 2005. Electrochemical evaluation of nucleoside analogue lamivudine in pharmaceutical dosage forms and human serum. Electroanalysis 17, 1886–1894. https://doi.org/10.1002/elan.200503307

Drug bank online, Efavirenz [WWW Document], n.d. URL https://go.drugbank.com/drugs/DB00625

Drug bank online, Lamivudin [WWW Document], 2024. URL https://go.drugbank.com/drugs/DB00709 (accessed 7.22.24).

Drug bank online, Tenofovir disoproxil fumarate [WWW Document], n.d. URL https://go.drugbank.com/drugs/DB00300

Godela, R., Kammari, V., Gummadi, S., Beda, D., 2021. Concurrent estimation of lamivudine, tenofovir disoproxil fumarate, and efavirenz in blended mixture and triple combination tablet formulation by a new stability indicating RP-HPLC method. Futur. J. Pharm. Sci. 7. https://doi.org/10.1186/s43094-021-00249-9

Gupta, S., Kesarla, R., Chotai, N., Omri, A., 2017. Development and validation of reversedphase HPLC gradient method for the estimation of efavirenz in plasma. PLoS One 12, 1–12. https://doi.org/10.1371/journal.pone.0174777

Kano, E.K., Serra, C.H.D.R., Koono, E.E.M., Andrade, S.S., Porta, V., 2005. Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies. Int. J. Pharm. 297, 73–79. https://doi.org/10.1016/j.ijpharm.2005.03.002

Medicines, F.H.I. V, 2024. FDA-Approved HIV Medicines HIV Overview 20–23.

Miedzinski, J., 1992. Early clinical signs and symptoms of HIV infection. Can. Fam. Physician 38, 1401–1410.

More, S.J., Tandulwadkar, S.S., Nikam, A.R., Rathore, A.S., Sathiyanarayanan, L., Mahadik, K.R., 2013. Separation and Determination of Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz in Tablet Dosage Form by Thin-Layer Chromatographic-Densitometric Method. J. Planar Chromatogr. - Mod. TLC 26, 78–85. https://doi.org/10.1556/JPC.26.2013.1.12

Murugan, S., Debnath, S., Sikdar, P., M, N.B., 2017. Simultaneous estimation of tenofovir disoproxil fumarate , efavirenz and lamivudine in bulk and tablet dosage form by UV spectrophotometry Simultaneous Estimation of Tenofovir Disoproxil Fumarate , Efavirenz and

Lamivudine in Bulk and Tablet Dosage Form b.

Naproxen, D., Suspension, O., Rs, U.S.P.N., Rs, U.S.P.N., Detector, L.C., 2020. Suitability requirements 3–4.

Onah, J.O., Ajima, U., 2011. Available online http://www.ijddr.in Covered in Official Product of Elsevier, The Netherlands Spectrophotometric Determination of Tenofovir Disoproxil Fumarate After Complexation With Ammonium Molybdate And Picric Acid 3, 199–204.

Rani, T.S., Sujatha, K., Chitra, K., Jacob, D.M., Yandapalli, R., Manasa, D., Sushma, B., 2012. Research and Reviews : Journal of Pharmaceutical Analysis Spectrophotometric Method for Estimation of Tenofovir Disoproxil Fumarate in Tablets. Res. Rev. J. Pharm. Anal. 1, 21–24.

Reddy Yellampalli, S., Reddy Mallu, U., Shanmukha Kumar, J. V., Pallepogu, J., Sudheer Kumar Reddy, G., Reddy Govindinne, L., 2018. Development and validation of uplc method for determination of lamivudine impurity profile in tablets. Asian J. Chem. 30, 1065–1069. https://doi.org/10.14233/ajchem.2018.21171

Sentenac, S., Fernandez, C., 2003. S ensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography 793, 317–324.

Sharma, R., Mehta, K., 2010. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian J. Pharm. Sci. 72, 527–530. https://doi.org/10.4103/0250-474X.73926

Sonar, K. V, Sapkale, P., Jadhav, A., Deshmukh, T., Patil, S., 2017. DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF LAMIVUDINE IN TABLET DOSAGE FORM 9.

Taylor, P., Kandagal, P.B., Manjunatha, D.H., Seetharamappa, J., Kalanur, S.S., n.d. RP - HPLC Method for the Determination of Tenofovir in Pharmaceutical Formulations and Spiked Human Plasma Tenofovir in Pharmaceutical Formulations 37–41. https://doi.org/10.1080/00032710801910742

Thapliyal, N., Osman, N.S.E., Patel, H., Karpoormath, R., Goyal, R.N., Moyo, T., Patel, R., 2015. NiO-ZrO2 nanocomposite modified electrode for the sensitive and selective determination of efavirenz, an anti-HIV drug. RSC Adv. 5, 40057–40064. https://doi.org/10.1039/c5ra05286f

Vikram, A., Nath, L.K., Pani, N.R., 2011. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. J. Pharm. Anal. 1, 251–257. https://doi.org/10.1016/j.jpha.2011.08.001

Xiao, J., Shi, S., Yao, L., Feng, J., Zuo, J., He, Q., 2022. Fast and Ultrasensitive Electrochemical Detection for Antiviral Drug Tenofovir Disoproxil Fumarate in Biological Matrices. Biosensors 12, 1–16. https://doi.org/10.3390/bios12121123