EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 1
351-357
Year: 2017

DEVELOPM ENT AND VALIDATION OF A STABILITY INDICATING NEW RP-HPLC METHOD FOR THE DETERMINATION OF LAMIVUDDINE AND TENFOVIR DISOPROXIL FUMARATE AND EFAVIRENZ

P. Srinivasa Rao* SVRM College, Ch. Subrahmanya Sastry PBN College and Dokku Ravi Teja

Department of Chemistry, SVRM College, Nagaram, Guntur (Dist), Andhra Pradesh, India.

***Corresponding Author: Dr. P. Srinivasa Rao**
Department of Chemistry, SVRM College, Nagaram, Guntur (Dist), Andhra Pradesh, India.

Article Received on 05/11/2016 **Article Revised on 25/11/2016** **Article Accepted on 15/12/2016**

ABSTRACT
A Simple High performance liquid chromatographic method (HPLC) was developed for the Analysis of Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz drug was carried out by 260 nm wave length. This method was found 99% of accuracy. According to International conference on Harmonization (ICH) guidelines Analytical parameters such as Linearity, accuracy, LOD, LOQ, Precision, Robustness, Rugdness, Forced Degradation and Stability have been established for the method for Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz tablets and statically to assess the application of the method. It can be successfully applied for the best analysis of Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz drug.

KEYWORDS: Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz drug and formulation, RP HPLC Method, Method validation.

INDRUDUCTION

Lamivudine is an antiretroviral medication used to prevent and treat HIV/AIDS and used to treat chronic hepatitis. It is of the nucleoside analog reverse transcriptase inhibitor (NRTI) class. Lamivudine chemically known as 4-amino-1-{(2R-,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl}-1,2-dihydropyrimidin-2-one.(Figure:1).Molecular formula is $C_8H_{11}N_3O_3S$.

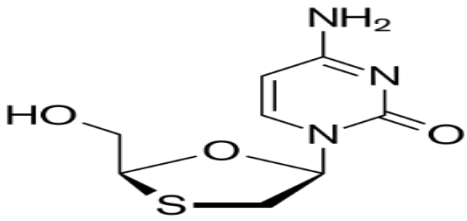

Fig. 1: Chemical Structure of Lamivudine

Tenofovir disoproxil Fumarate is an antiretroviral medication used to prevent and treat HIV/AIDS and to treat chronic hepatitis B. The active substance is tenofovir, while tenofovir disoproxil isa prodrug that is used because of its better absorption in the gut. Tenofovir disoproxil chemically known as Bis{[(isopropoxycarbonyl)oxy]methyl}({[(2*R*)-1-(6amino-9*H*-purin-9-yl)-2propanyl]oxy}methyl)phosphonate (Figure: 2). Molecular formula $C_{19}H_{30}N_5O_{10}P$.

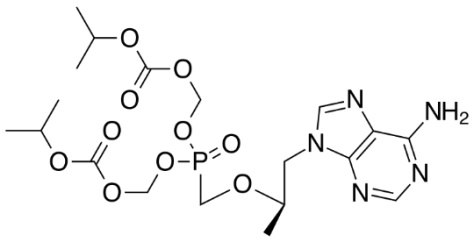

Fig. 2: Chemical Structure of Tenfovir

Efavirenz It is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type 1. For HIV infection that has not previously been treated. Efavirenz chemically known as (4*S*)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H*-3,1-benzoxazin-2-one (Figure:3). Molecular formula $C_{14}H_9ClF_3NO_2$.

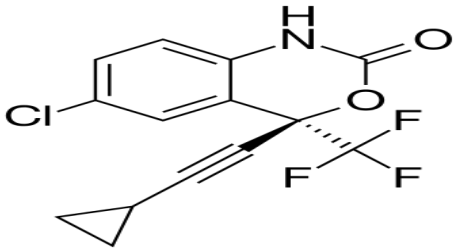

Fig. 3: Chemical Structure of Efavirenz

In Most of researchers are reported different HPLC methods they are use many different substances have been used for buffering in HPLC mobile phase, but no reports on the simple buffers like water. In this paper, we reported the development and validation of accurate HPLC method for analysis bulk and formulations of Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz.

MATERIALS AND METHODS

Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz drug and tablets was purchased in Cipla, Bangalore. HPLC grade methanol, acetonitrile and HPLC water were purchase from merk company.

EXPERIMENTAL CONDITIONS

A high performance liquid chromatograph system, with Waters-Alliance system, with an auto sampler the analysis. The data was recorded using Empower software. The samples separation was performed on a Agilent C18, (5 µ, 250 mm x 4.6 mm) with the mobile phase consisting of acetonitrile and water with ratio 50:50 at ambient temperature. The flow rate was kept at 1.0 ml/min and the determination wavelength was 260 nm.

Preparation of standard Solution

300 mg of Lamivudine, 300 mg of Tenofovir Disoproxil Fumarate and 600 mg of Efavirenz working standards into a 100 mL volumetric flask of mobile phase. Now 5 ml of this solution was taken from above solution and again it dissolved in 50ml of mobile phase and it is known the standard solution.

Preparation of Sample solution

Weigh accurately 10 tablets and crush to powder take 5 tablets equivalent weight of sample into a 500 ml volumetric flask. Add 350 ml of diluents, sonicate for 30minutes to dissolve and dilute to volume diluent. Further dilute 5ml to 50ml with the diluents. Filter through 0.45µ nylon syringe filter.

Validation

Linearity was determined by injecting different concentration of sample solutions. For system precision, standard solution was injected to six replicates injections to check %RSD (relative standard deviation) and for method precision six time sample were prepared and each of those were prepared and each of those were injected in duplicate. Mean of all of these values gives to assay value.

To establish the within-day (intra-assay) and between-day (inter-assay) accuracy and precision of the method, Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz was assayed on one day and three separate days. Intra-assay and inter-assay were calculated.

Accuracy perform the recovery study by adding known concentration of working standard in the range of 50% to 150% of specification limit. Report the % recovery in presence of samples.

Limit of detection (LOD) estimating the lowest concentration of standard qualitatively by injecting six times very low concentration of standard in trace levels during cleaning validation.

Limit of Quantitation (LOQ) estimating the lowest concentration of standard qualitatively by injecting six times very low concentration of standard in trace levels during cleaning validation.

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate (±20%), organic content in mobile phase (± 2%), wavelength of detection (± 5%). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

Forced degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. The degradation peaks should be well separated from each other and the resolution between the peak should be at least 1.0 and the peak purity of the principal peaks shall pass.

Stability by preparaing the analytical solution and injecting at periodic intervals of 24 hours to 48 hours at 3 to 4 hour intervals depending on the instrument utilization and sequence of injection.

RESULTS AND DISCUSSION

In this paper, we developed the reverse phased column procedure for a suitable method for the pharmaceutical analysis of Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz drug and tablets. A typical chromatogram obtained (Fig-4) by using the mobile phase. The precission, accuracy and forced degradation of the method was determined from Lamivudine and Tenfovir Disoproxil Fumarate and Efavirenz dosage form and obtained. Inter and intra- day studies were performed in three concentrations of the drug was reported on three consecutive days.

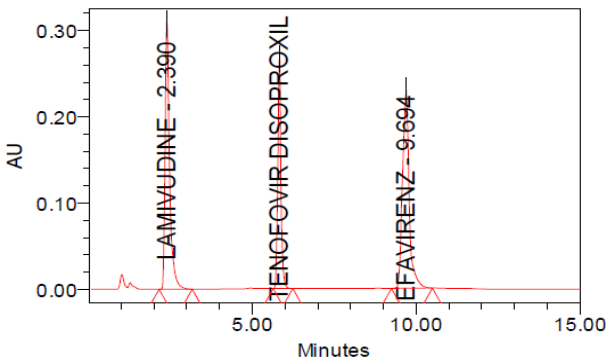

Fig.4-Typical Chromatogram

METHOD VALIDATION

The method was validated for linearity, precision, accuracy, robustness, rugdness, forced degradation and stability of the method was studied by the Lamivudine, Tenfovir and Efavirenz.

Linearity was prepared in the range of 60-450 µg/ml, 60-450 µg/ml and 20-900 µg/ml solutions are analyzed through the high pressure liquid chromatographic technique. The peak area were plotted against concentration was subjected to linear plots (Fig:5,6 and 7).

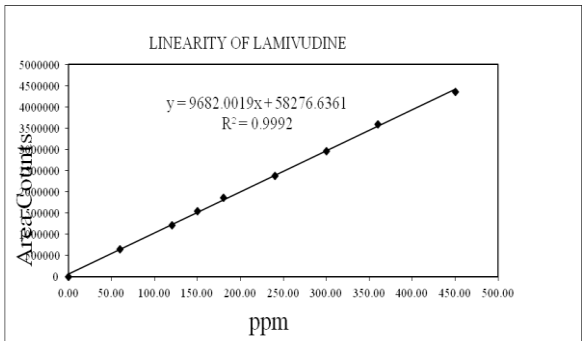

Fig: 5 Linearity plot for Lamivudine

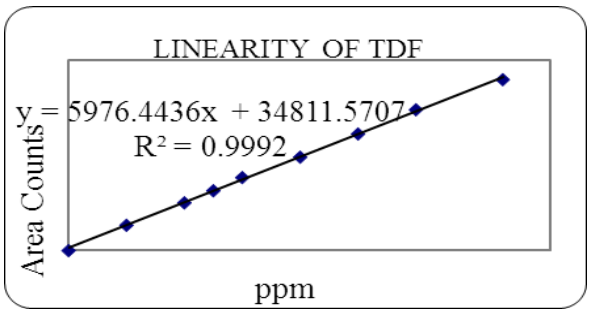

Fig: 6 Linearity plot for Tenfovir

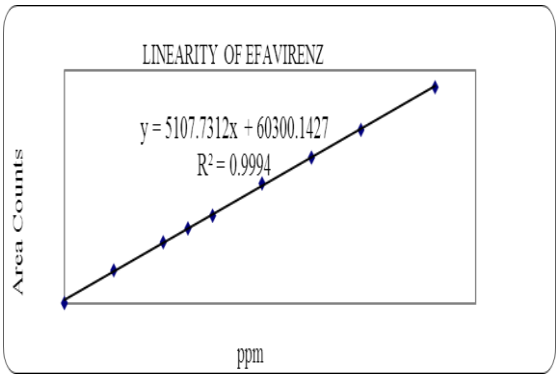

Fig: 7 Linearity plot for Efavirenz

Precision of this method was studied in inter day and intra day variation. The precision of intraday studies of three different concentration of the drug was repeated thrice in a day and in the inter day variation studies of three different concentration of the drug was repeated on three consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra-day precision studies were found to be less than 2%. Good recoveries (98 - 100%) of the drug were obtained at each added concentration, indicating that the method was accurate. These results are represented in Table 1,2 and Table 3 and chromatograms are shown in figure nos-8,9,10. Accuracy and percentage of drug recovery were calculated from chromatographic methods and the data is shown in Table 4,5 and Table-6 and chromatograms are shown in figure nos-11,12 and 13.

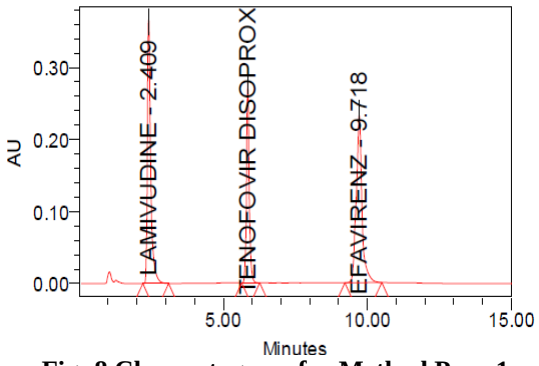

Fig: 8 Chromatogram for Method Prec-1

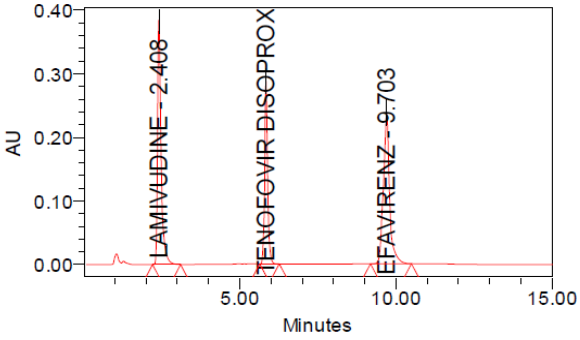

Fig: 9 Chromatogram for Method Prec-2

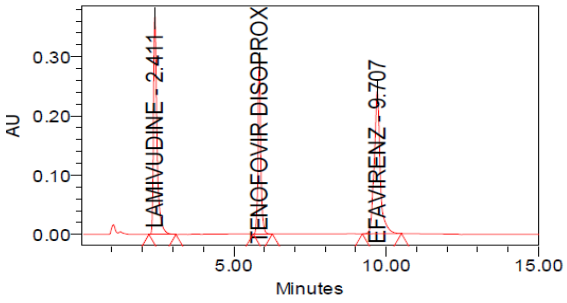

Fig: 10 Chromatogram for Method Prec-3

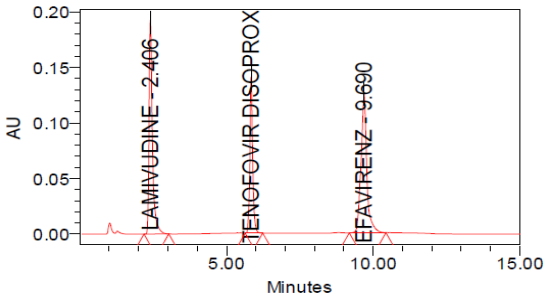

Fig: 11 Chromatogram for Accu- 50%

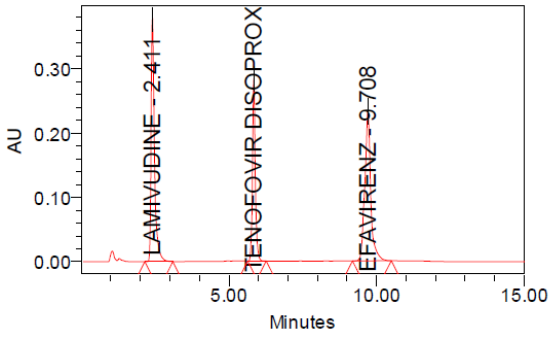

Fig: 12 Chromatogram for Accu- 100%

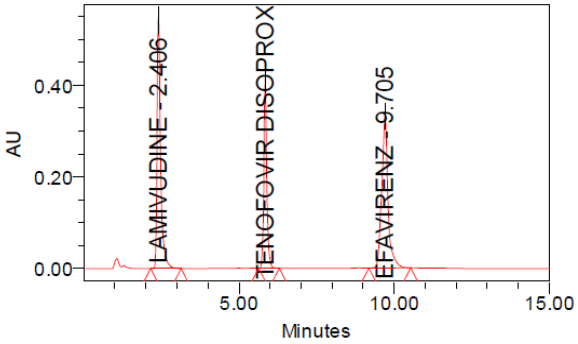

Fig: 13 Chromatogram for Accu- 150%

Table 1: Precision data of Lamivudine Intra day

Concentration of Lamivudine drug mg.ml	Area mAU	Relative standard deviation (RSD)
300	2967776	0.84
300	3088817	0.68
300	2962033	1.02

Table 2: Precision data of Tenfovir Intra day

Concentration of Tenfovir drug mg.ml	Area mAU	Relative standard deviation (RSD)
300	1827803	0.08
300	1887496	0.15
300	1826955	0.68

Table 3: Precision data of Efavirenz Intra day

Concentration of Efavirenz drug mg.ml	Area mAU	Relative standard deviation (RSD)
600	3225539	0.72
600	3286920	0.66
600	3262277	0.78

LOD and LOQ minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be Lamivudine, Tenfovir and Efavirenz drugs are 0.30 and 3.00 µg/ml, 0.30 and 3.00 µg/ml and 0.60 and 6.00 µg/ml, respectively. The chromatographic data as shown below fig. no's 14 and 15.

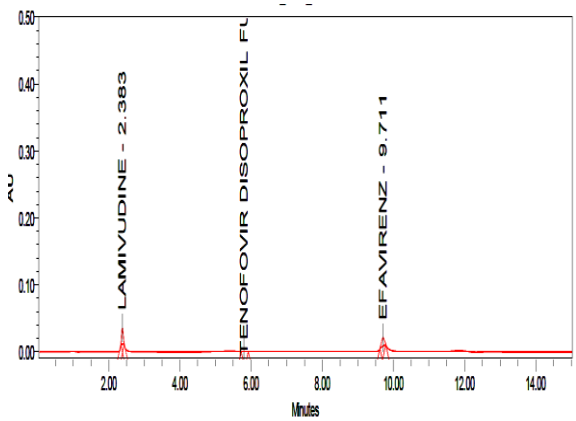

Fig: 14 Chromatogram for LOD

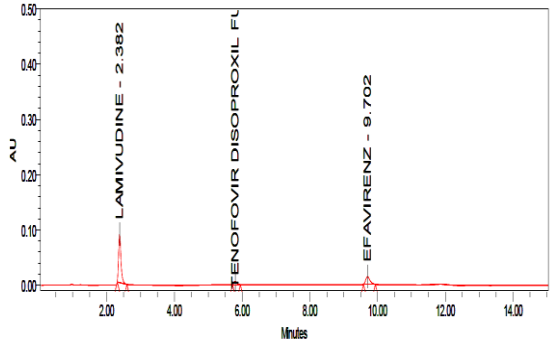

Fig: 15 Chromatogram for LOQ

Forced degradation study was observed that upon treatment of Lamivudine, Tenfovir and Efavirenz with different strengths of base (0.05 N and 0.5 N NaOH), acid (0.05 N, 0.5 N and 1 N HCl) and hydrogen peroxide and Thermal and Photolytic (20%) the degradation was observed in (Table 7). Further it is important to note that from the chromatograms (Figure 16 to 20), it is evident that although the degraded peaks are observed. The Lamivudine, Tenfovir and Efavirenz stable under the applied stress conditions like Thermal, acid and alkaline and oxidative degradation states.

Stress condition/duration/solution	Degradation
Acid degradation (0.5 N HCl, 1 hr)	25%
Alkaline degradation (0.5 N NaOH, 1 hr)	22%
Oxidative degradation (30 % H2O2,80°C for 10 min)	28%
Thermal degradation (Solid sample, 80°C, 3 hr)	23%
Photolytic Degradation (sample expose sun light 6 hr)	27%

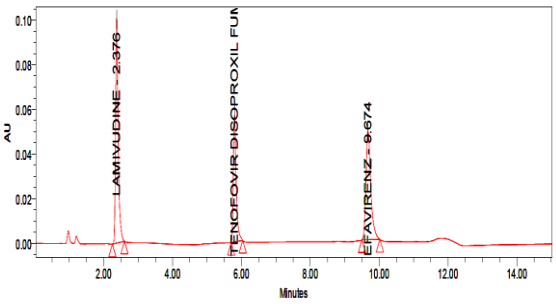

Fig: 16 Chromatogram for Acid Deg.

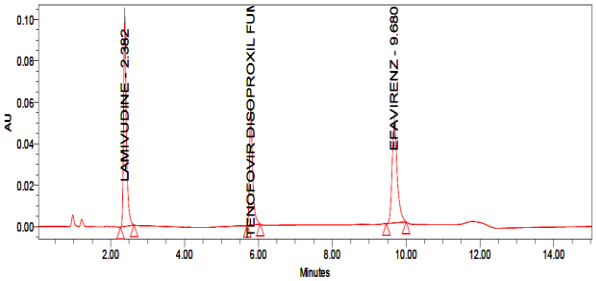

Fig: 17 Chromatogram for Alkali Degr.

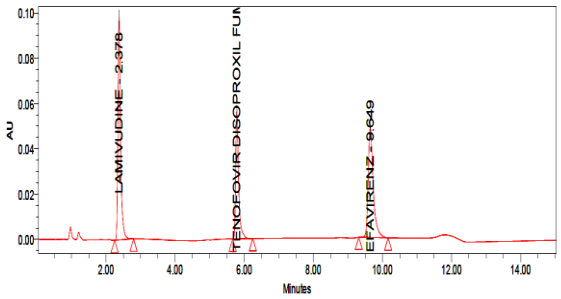

Fig: 18 Chromatogram for Peroxide Degr.

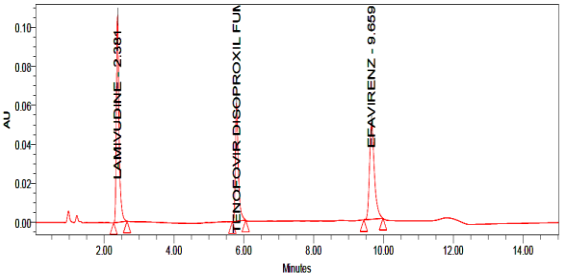

Fig: 19 Chromatogram for Thermal Degra.

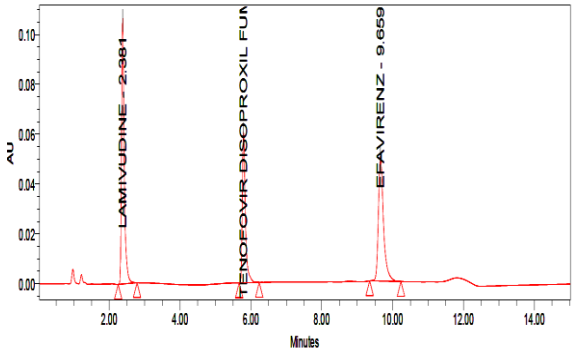

Fig: 20 Chromatogram for Photolytic Degradation

Robustness of the method small changes in chromatographic conditions such as change in flow rate (± 20%), organic content in mobile phase (± 2%), wavelength of detection (± 5%) studied to determine the robustness method for the analysis of Lamivudine, Tenfovir and Efavirenz. The influence of changes in chromatographic parameters are shown in table no.8. The chromatographic data fig. no's 21 and 15.

Table No.:8 Results for Robustness study

Change in parameter	% RSD
Flow (0.8 ml/min)	0.65
Flow (1.2 ml/min)	0.92
Wavelength (265 nm)	0.74
Wavelength (255 nm)	0.56
Organic phase composition (+2%)	0.24
Organic phase composition (-2%)	0.22

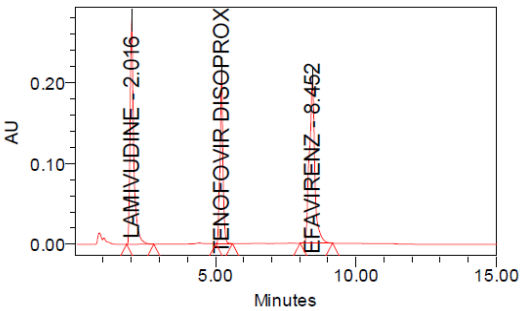

Fig: 21 Chromatogram for Flow Plus

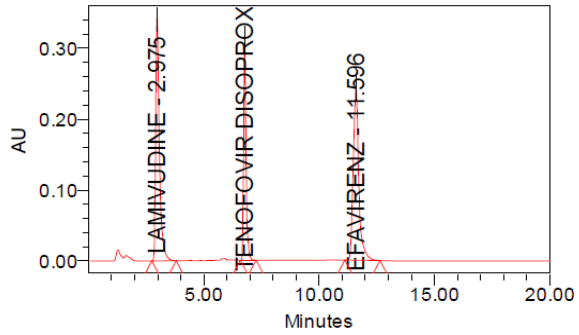

Fig: 22 Chromatogram for Flow Minus

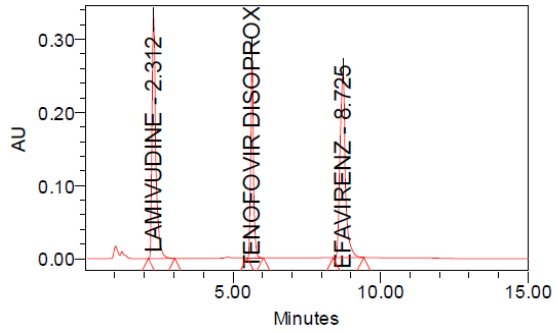

Fig: 23 Chromatogram for Org Plus

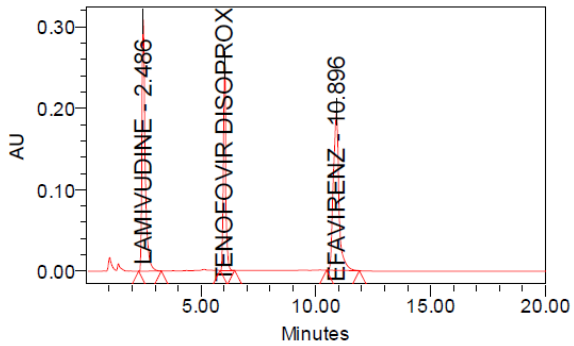

Fig: 24 Chromatogram for Org Minus

CONCLUSIONS

The developed method is accurate, precise and reliable for the analysis of Lamivudine, Tenfovir and Efavirenz in pharmaceutical formulations. This method was validated for linearity, accuracy, precision, robustness of Lamivudine, Tenfovir and Efavirenz drug. The RSD values for all parameters were found to be less 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used for better analysis and pharmaceutical formulations of Lamivudine, Tenfovir and Efavirenz drug.

REFERENCES

1. Appala et al, 2008 Appala, N.R., Rao, V.J., Vanitha, P.K., 2008. Mukilteo, K., Srinivasu, K., Simultaneous Estimation of Tenofovir Disoproxil, Emtricitabine and Efavirenz in Tablet Dosage Form by RP- HPLC. Orient. J. Chem. 24(2), in press.
2. Budawari, 2001, S. Budawari The Merck Index (Thirteenth ed.)Merck and Co. Inc., Whitehouse Station, NJ, 2001; 630: 1631-32.
3. Deirdre Fox et al., 2011 B.C. Deirdre Fox, D. Robert O'Connor, A. Patrick Mallon, Gillian McMahon Simultaneous determination of efavirenz, rifampicin and its metabolite desacetyl rifampicin levels in human plasma Pharmaceut. Biomed. Anal., 2011; 56: 785–791.
4. Gish et al., 2005, R.G. Gish, H. Trinh, N. Leung, F.K.L. Chan, M.L. Fried, T.L. Wright, C. Wang, J. Anderson, E. Mondou, A. Snow, J. Sorbel, F. Rousseau, L. Corey Safety and antiviral activity of emtricitabine (FTC) for the chronic hepatitis B infection: a two-year study J. Hepatol., 2005; 43(1): 60–66 Article, PDF (151 K) View Record in Scopus Citing articles (87).
5. ICH Q2A, 1994 ICH Q2A Text on Validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 Oct 1994.
6. ICH Q2B, 1996, ICH Q2B Text on validation of analytical procedures: Methodology International Conference on Harmonization Nov. 1996.
7. Joshi et al., 2009, M. Joshi, A.P. Nikalje, M. Shahed, M. Dehghan HPTLC Method for the Simultaneous Estimation of Emtricitabine and Tenofovir in Tablet Dosage Form Ind. J. Pharmaceut. Sci., 2009; 71: 95–97 View Record in Scopus Citing articles (16).
8. Kandagal et al., 2008 P.B. Kandagal, D.H. Manjunatha, J. Seetharamappa, S.S. Kalanur RPHPLC Method for the Determination of Tenofovir in Pharmaceutical Formulations and Spiked Human Plasma Anal. Lett., 2008; 41(4): 561–570 Full Text via CrossRef View Record in Scopus Citing articles (27).
9. Laxman et al., 2010 V. Laxman, Potale, A.S. Khodke, S.M. Patole, M.C. Damle Development and Validation of Stability Indicating HPTLC Method for Determination of Efavirenz as Bulk Drug and in Pharmaceutical Formulation J. Adv. Pharmaceut. Res., 2010; 1(2): 115–122.
10. Malipatil and Nandedkar, 2009 S.M. Malipatil, M.A. Nandedkar Determination of Tenofovir Disoproxil Fumarate by a Sensitive Simple Isocratic RP-HPLC Method J. Ind. Council Chem., 2009; 26(1): 67–69 View Record in Scopus Citing articles (5).
11. Martindale, 2002 Martindale The Complete Drug Reference (Thirty third ed.)Pharmaceutical Press, London, 2002; pp. 620, 642.
12. Montgomery et al., 2001 E.R. Montgomery, A.L. Edmanson, S.C. Cook, P.K. Hovsepian Development and Validation of a Reverse-Phase HPLC Method for Analysis of Efavirenz and Its Related Substances in the Drug Substance and in a Capsule Formulation J. Pharmaceut. Biomed. Anal., 2001; 25(2): 267–284. Article PDF (161 K) View Record in Scopus Citing articles (22).
13. Physician's Desk Reference, 2008 Physician's Desk Reference, 2008. Sixty second ed. Thomson Healthcare Inc., Montvale, NJ, pp. 924.

14. Rao and Nikalje, 2009 B.U. Rao, A.P. Nikalje Stability- indicating HPLC method for the Determination of Efavirenz in Bulk Drug and in Pharmaceutical Dosage Form Afr. J Pharm. Pharmacol., 2009; 3: 643–650 View Record in Scopus Citing articles (15).
15. Rezk et al., 2005 N.L. Rezk, R.D. Crutchley, D. Angela, M. Kashuba Simultaneous quantification of emtricitabine and Tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction J. Chromatogr. B., 2005; 822: 201–208 Article PDF (228 K) View Record in Scopus Citing articles (74).
16. Rolim-Neto, 2011 Pedro José Rolim-Neto Development and validation of a HPLC Analytical Assay Method for Efavirenz Tablets: A Medicine for HIV Infections Braz. J. Pharm. Sci., 2011; 47: 97–102.
17. Sagar et al., 2009 S.P. Sagar, D. Pratik, M.M. Annapurna Stability Indicating Ion-Pair RP-HPLC Method for Estimation of Tenofovir Disoproxil Fumarate in Tablet Dosage Forms Int. J. Pharmaceut. Res. Dev., 2009; 1: 1–9 View Record in Scopus Citing articles (1).
18. Sharma and Pooja Gupta, 2009 Rajesh Sharma, A. Pooja GuptaValidated RP - HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form Eurasian J. Anal. Chem., 2009; 4(3): 276–284 Full Text via CrossRef View Record in Scopus Citing articles (12).
19. Shirkhedkar Atul et al., 2009 H. Shirkhedkar Atul, J. Bhirud Charushila, S. Surana Application of UV-Spectrophotometric Methods for Estimation of Tenofovir Disoproxil Fumarate in Tablets Pak. J. Pharm. Sci., 2009; 22(1): 27–2.
20. Sockalingam et al., 2005 A. Sockalingam, Indumathy Narayanareddy, Pitchaimuthu Shanmugapandiyan, S.K. Sridhar Simultaneous Quantification of Stavudine, Lamivudine and Nevirapine by UV Spectroscopy, Reverse Phase HPLC and HPTLC in Tablets J. Pharmaceut. Biomed. Anal., 2005; 39(3–4): 801–804 View Record in Scopus Citing articles (1).