Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (3):148-155
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

RP-HPLC method for simultaneous estimation of lamivudine, didanosine and efavirenz in pharmaceutical dosage forms

B. Naga Sandhya, A. Manikanta Kumar, Mahesh K Nasare, P. Vijay Kumar, Jetta Satish and Prakash. V. Diwan.

Department of Pharmaceutical Analysis and Quality Assurance, School of Pharmacy, Anurag Group of Institutions, Venkatapur (V), Ghatkesar (M), Rangareddy (D)

ABSTRACT

A simple, specific, accurate and precise reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of lamivudine, didanosine and efavirenz in pharmaceutical dosage form. The chromatographic separation for lamivudine, didanosine and efavirenz was achieved with mobile phase containing water: tetrahydrofuran: acetonitrile (45.83: 20.83: 33.34 % v/v/v), Oyster BDS premium C18 (250 mm × 4.6 mm, 5 µm) analytical column in isocratic mode at room temperature and UV detection at 245 nm. The compounds were eluted at a flow rate of 1.15 ml min^{-1}. The retention times of lamivudine, didanosine and efavirenz were found to be 2.01 ± 0. 003, 3.01 ± 0.001 and 8.61 ± 0.002 min respectively . Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness were determined according to ICH guidelines. The linear dynamic ranges were obtained from 15-90, 12.5-75 and 30-180 µg mL^{-1} for lamivudine, didanosine and efavirenz respectively. The mean recoveries obtained for lamivudine, didanosine and efavirenz were 99.85, 99.78 and 99.94 % respectively. Limit of detection and quantification for lamivudine 0.61 and 1.85 µg mL^{-1}, for didanosine 0.43 and 1.31 µg mL^{-1} and for efavirenz 0.65 and 1.97 µg mL^{-1} respectively. The developed method was free from interferences due to excipients present in formulation and it can be used for routine quality control analysis.

Key words: lamivudine; didanosine; efavirenz; HPLC; Validation

---

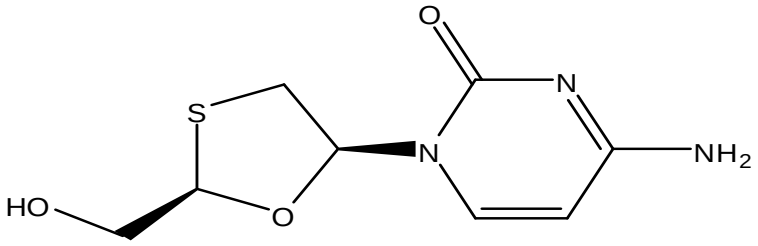
INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs, which are introduced as antiretroviral agents for the treatment of infection with human immune deficiency virus (HIV). Additional drug classes were developed. They are protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors. Lamivudine is nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus Fig. 1(a). Nucleoside reverse transcriptase inhibitors (NRTIs) are the prodrugs that require intracellular phosphorylation to their corresponding triphosphate derivatives, which are the active inhibitors of HIV reverse transcriptase [1, 2]. Didanosine is nucleoside reverse transcriptase inhibitor with activity against human immune deficiency virus (HIV) and hepatitis B virus Fig. 1(b). It acts as a chain terminator of viral DNA [3, 4].

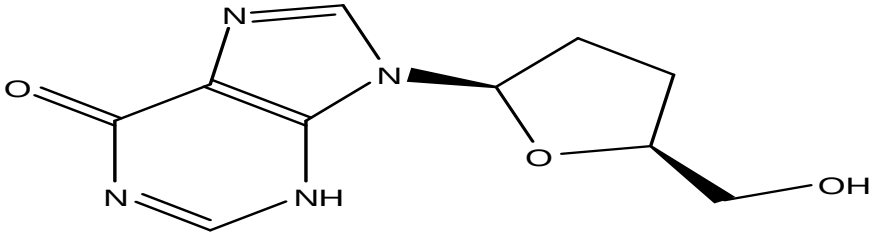
Efavirenz is non-nucleoside reverse transcriptase inhibitors. It is used in the treatment of HIV infection Fig. 1(c). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme [5, 6]. Literature survey reveals that there are no isocratic RP-HPLC methods for the simultaneous estimation of lamivudine, didanosine and efavirenz in the bulk drugs and in the formulation. Individual methods have been reported for the determination of lamivudine, didanosine and efavirenz in pharmaceutical dosage forms, biological fluids or in combination with other

drugs [7-18] and two gradient methods are available for the simultaneous estimation of lamivudine, didanosine and efavirenz in human plasma [19-20]. To our knowledge, no study related to the isocratic analysis of lamivudine, didanosine and efavirenz have been reported in literature. Therefore, there is a challenge to develop isocratic RP-HPLC method for the simultaneous estimation of lamivudine, didanosine and efavirenz. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical and precise isocratic RP-HPLC method for the simultaneous estimation of three drugs in pharmaceutical dosage form.

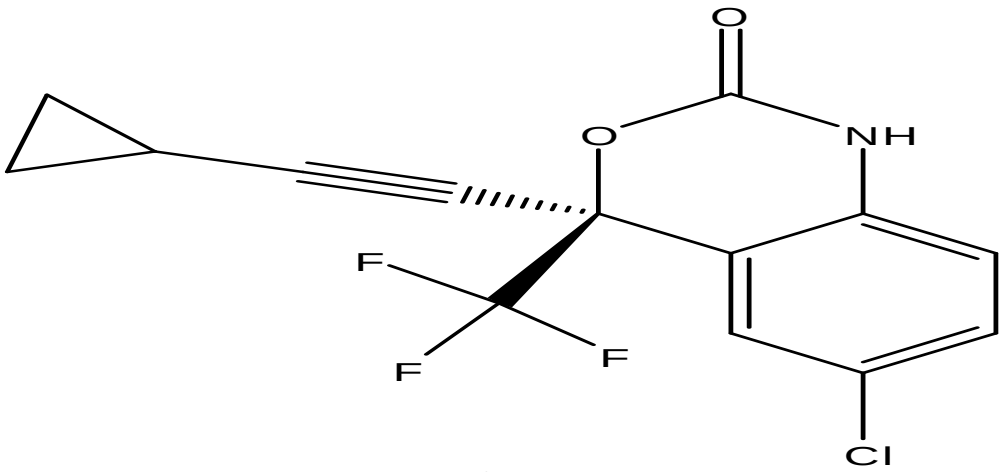
Fig. 1 Structures of (a) lamivudine, (b) didanosine (c) efavirenz



(a) lamivudine



(b) didanosine



(c) efavirenz

MATERIALS AND METHODS

2.1. Materials

Acetonitrile (HPLC grade) and water (HPLC grade) were obtained from Merck specialities private limited, Mumbai, India. Tetra hydro furan was obtained from Rankem analytical reagent, New Delhi, India. The drug samples of Lamivudine, Didanosine and Efavirenz were received as a gift samples from CIPLA LTD. Odivir-250 kit (Label claim: lamivudine 300 mg, didanosine 250 mg and efavirenz 600 mg) was manufactured by Cipla and purchased from Apollo pharmacy, Hyderabad, India.

2.2. Method development and optimization of chromatographic conditions

A Waters® HPLC system was used for the analysis. The column used for chromatographic separations was Oyster BDS premium C18 (250 mm × 4.6 mm, 5 µm). The chromatographic separation for lamivudine, didanosine and efavirenz was achieved with mobile phase containing water: tetra hydro furan: acetonitrile (45.83: 20.83: 33.34 % v/v/v), filtered through 0.45 µm filter using value 1 stage vacuum pump and deaerated in ultra sonic bath sonicator. The analytical wave length was set at 245 nm. Mobile phase was pumped in isocratic mode at a flow rate of 1.15 ml min^{-1}. The separation was carried out at room temperature.

2.3. Preparation of standard solutions

Stock solutions were prepared by dissolving 30 mg of lamivudine, 25mg of didanosine and 60 mg of efavirenz were accurately weighed and dissolved in 50 ml of mobile phase. Aliquots of the standard stock solutions of lamivudine, didanosine and efavirenz were transferred into 100 ml volumetric flasks and the solutions were made up to volume with mobile phase to obtain final concentrations of 15, 30, 45, 60, 75, 90 µg mL^{-1} for lamivudine, 12.5, 25, 37.5, 50, 62.5, 75 µg mL^{-1} for didanosine and 30, 60, 90, 120, 150, 180 µg mL^{-1} for efavirenz.

2.4. Preparation of sample solution for assay

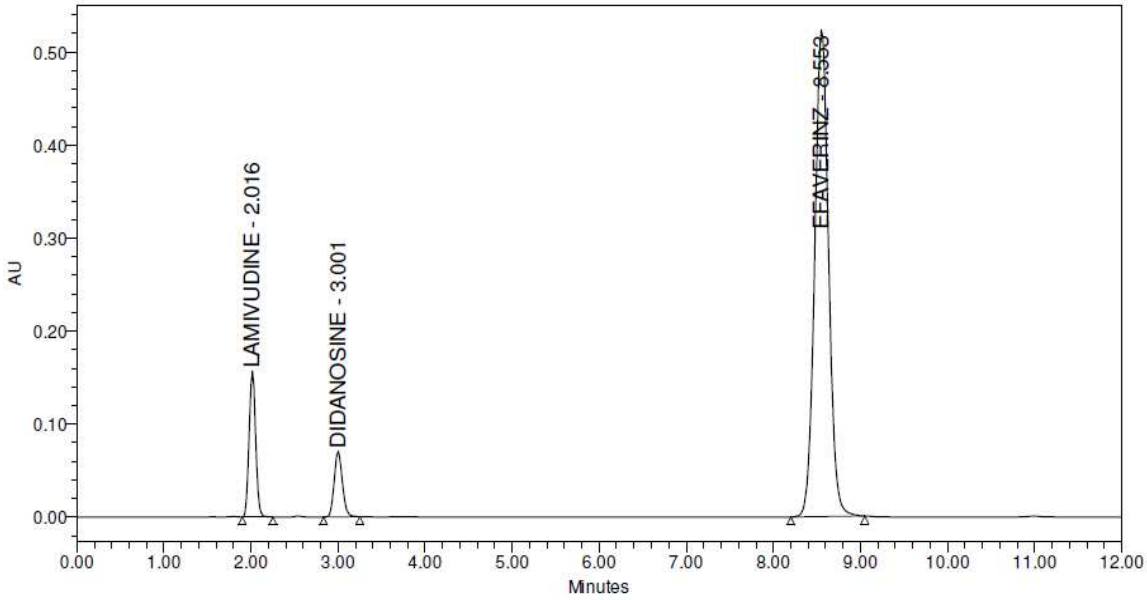
Twenty Odivir-kits each containing 300 mg lamivudine, 250 mg didanosine and 600 mg efavirenz were weighed, average weight was calculated and powdered. A quantity equivalent to 300 mg of lamivudine, 250 mg of didanosine and 600 mg of efavirenz was weighed and transferred into 100 ml volumetric flask. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered. Suitable aliquots of formulation were prepared and injected into HPLC to obtain concentration of the three drugs in the linearity range.

RESULTS AND DISCUSSION

3.1. HPLC method development and optimization

An Oyster BDS premium C18, 250×4.6 mm, 5 µm, i.d., and column maintained at ambient temperature was used for the separation and the method was validated for the estimation of lamivudine, didanosine and efavirenz in Odivir-250 kit. The composition, pH and the flow rate of the mobile phase were optimized. A mobile phase consisting of water: tetra hydro furan: acetonitrile (45.83: 20.83: 33.34 % v/v/v), set at a flow rate of 1.15 ml min^{-1} was selected for use for further studies after several preliminary investigatory chromatographic runs. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in Fig. 2. Under the described experimental conditions, all peaks were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition, and flow rate were evaluated as a part of testing for method robustness.

Fig. 2 A typical chromatogram of standard lamivudine (60 µg mL^{-1}), didanosine (50 µg mL^{-1}) and efavirenz (120 µg mL^{-1}) measured at 245 nm



3.2. Validation of the method

The analytical method was validated with respect to parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness and ruggedness [21].

3.2.1. Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 15-90 µg mL^{-1} for lamivudine, 12.5-75 µg mL^{-1} for didanosine and 30-180 µg mL^{-1} for efavirenz. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficients were found to be 0.9994, 0.9992 and 0.9996 for lamivudine, didanosine and efavirenz respectively. The results are given in Table 1.

Table 1: Linear regression data for calibration curves

Parameters	Lamivudine	Didanosine	Efavirenz
Linearity range (µg mL^{-1})	15-90	12.5-75	30-180
Correlation coefficient	0.9994	0.9992	0.9996
Slope	14273	9653.3	50464
Intercept	32490	-21559	-205508

3.2.2. Precision

Intra-day precision was investigated by injecting six replicate samples of each of the samples on the same day. The % RSD obtained for lamivudine, didanosine and efavirenz were found to be 0.006, 0.02 and 1.29 respectively. Inter-day precision was assessed by injecting the same three samples over six consecutive days. The % RSD obtained for lamivudine, didanosine and efavirenz were found to be 0.354, 1.24 and 0.04 respectively. The results are given in Table 2.

Table 2: Precision studies

Intra-day precision		Inter-day precision	
Drugs	% *RSD	Drugs	% *RSD
Lamivudine	0.006	Lamivudine	0.354
Didanosine	0.02	Didanosine	1.24
Efavirenz	1.29	Efavirenz	0.04

**mean of six observation*

3.2.3. Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of standard lamivudine, didanosine and efavirenz corresponding to 50, 100 and 150% of label claim had been added. At each level of the amount three determinations were performed. The mean recoveries obtained for lamivudine, didanosine and efavirenz were 99.85, 99.78 and 99.94 %, respectively. The results are given in Table 3.

Table 3: Accuracy studies

Drugs	Amount taken (mg)	Amount added (mg)	Total amount found (mg)	% Recovery	% RSD
Lamivudine	300	150	149.42	99.85	0.461
		300	300.16		
		450	448		
Didanosine	250	125	124.72	99.78	0.624
		250	248.17		
		375	374.40		
Efavirenz	600	300	299	99.94	0.546
		600	598.23		
		900	895.13		

**mean of six observations*

3.2.4. Specificity

The method specificity was assessed by comparing the chromatograms obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in water without drug), acid and alkaline conditions. The method was specific as none of the excipients interfered with the analytes of interest Fig. 3.

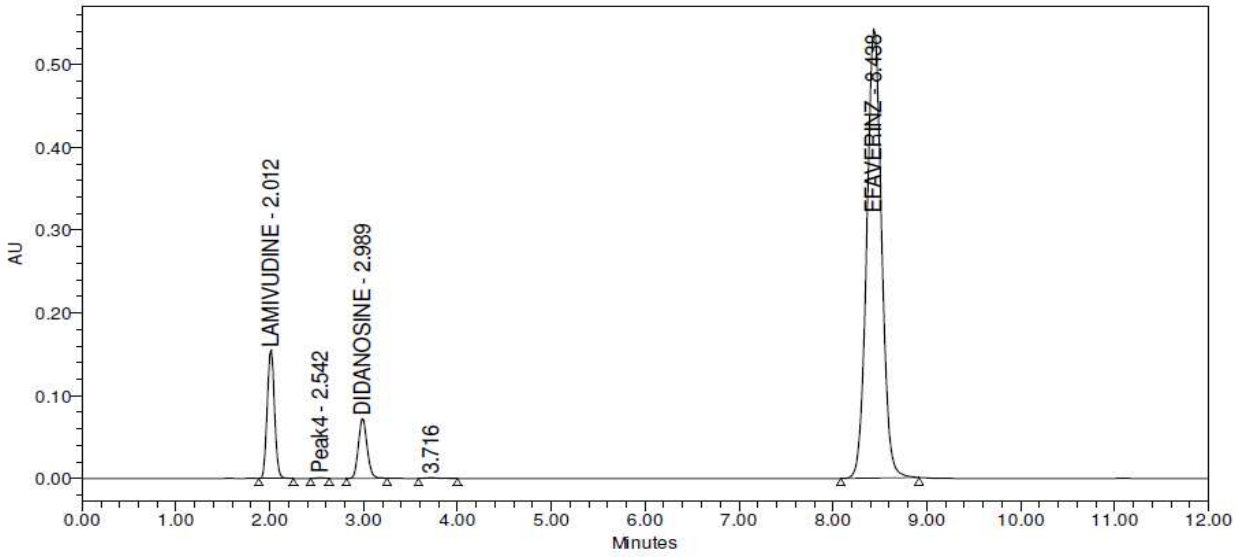
3.2.5. LOD and LOQ

LOD and LOQ of lamivudine, didanosine and efavirenz were determined by calibration curve method. LOD and LOQ for lamivudine were 0.61 and 1.85 µg mL^{-1}, for didanosine were 0.43 and 1.31 µg mL^{-1} and for efavirenz were 0.65 and 1.97 µg mL^{-1}. The results are given in Table 4.

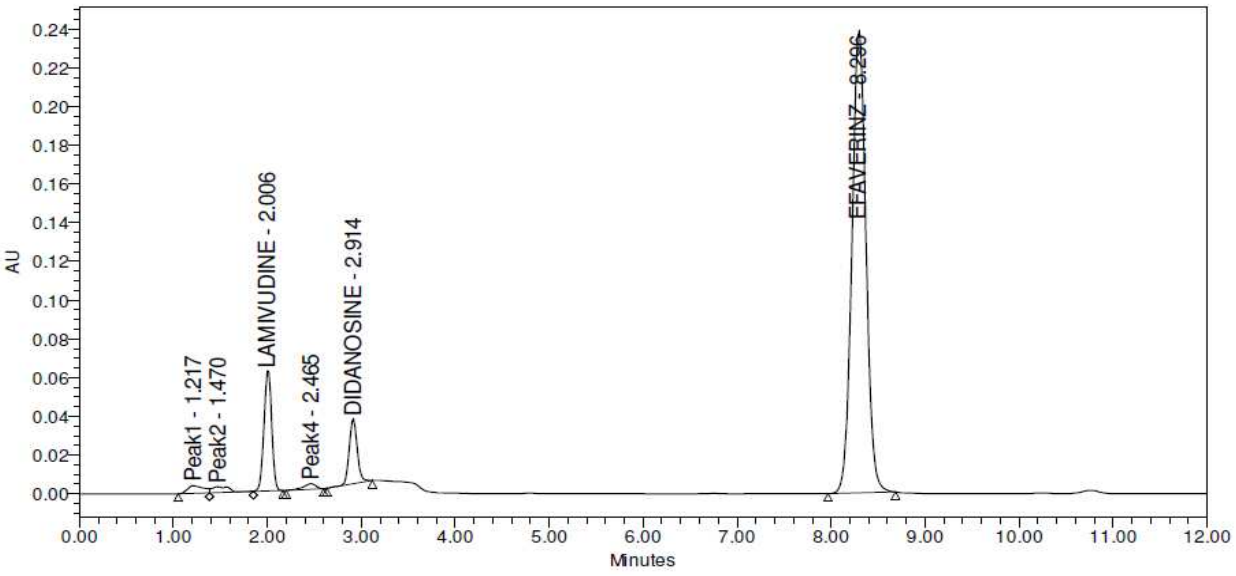
Table 4: LOD and LOQ studies

Drugs	Limit of detection (LOD) µg mL^{-1}	Limit of Quantification (LOQ) µg mL^{-1}
Lamivudine	0.61	1.85
Didanosine	0.43	1.31
Efavirenz	0.65	1.97

Fig. 3 Specificity chromatograms of lamivudine, didanosine and efavirenz

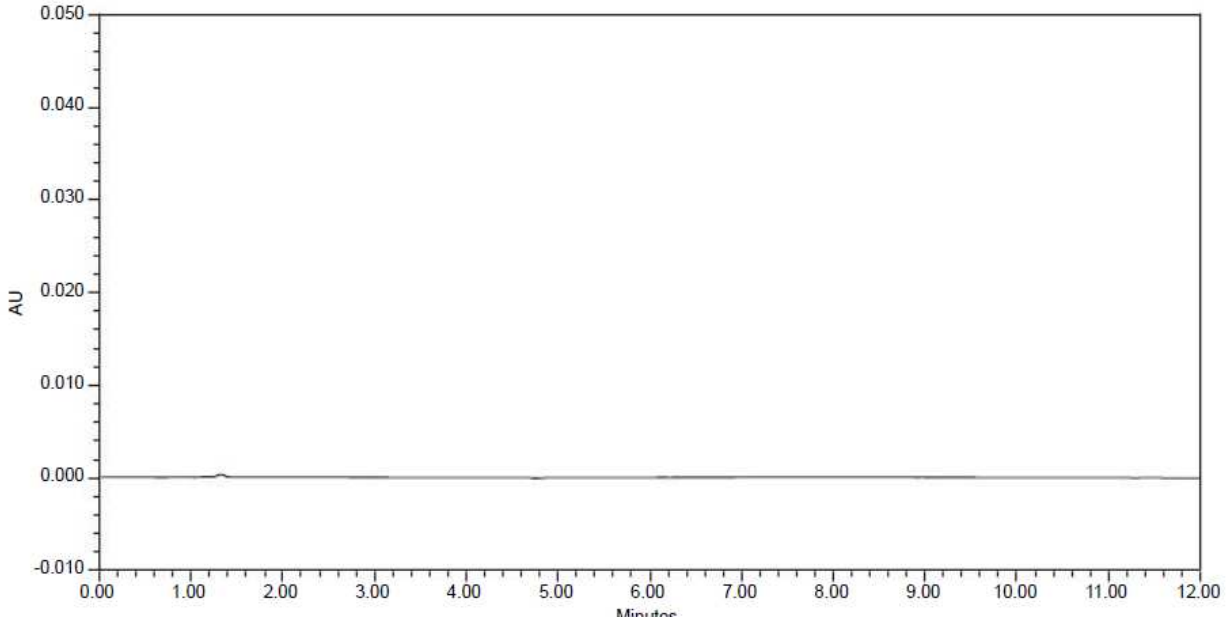


Base Conditions

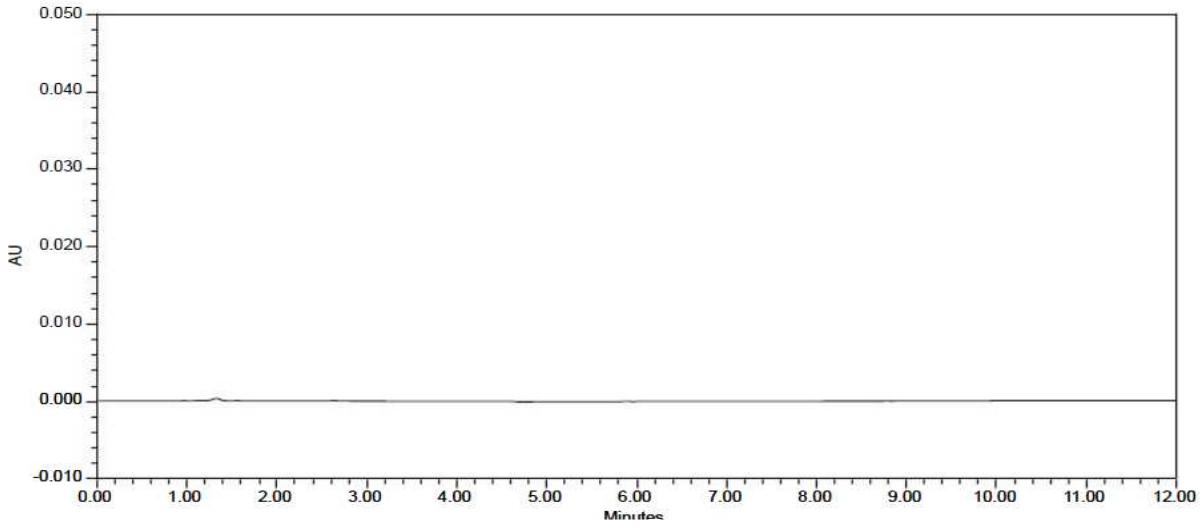


Acid Conditions

Cont….



Placebo Conditions



Blank

3.2.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. The results are given in Table 5.

Table 5: Robustness studies

Parameters	Retention time (min)		
Flow rate (ml min^{-1})	Lamivudine	Didanosine	Efavirenz
1.10	2.151	3.204	9.103
1.15	2.016	3.001	8.553
1.20	1.894	2.813	7.960

3.2.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for lamivudine, didanosine and efavirenz that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method. The results are given in Table 6.

Table 6: Ruggedness studies

Drugs	Peak area		% *RSD
	Analyst 1	Analyst 2	
Lamivudine	817554	817878	0.7456
Didanosine	457543	456393	0.9687
Efavirenz	5822165	5816653	1.0234

** mean of six observations*

3.3 Analysis of marketed formulation

The proposed RP-HPLC method was applied to the simultaneous estimation of lamivudine, didanosine and efavirenz in Odivir-250 kit and drug content in each sample were calculated by comparison with the appropriate standard solution of the drug. No interference due to excipients was detected in the chromatograms produced Fig. 4. Satisfactory results were given in Table 7.

Fig. 4 A typical chromatogram of formulation lamivudine (60 µg mL^{-1}), didanosine (50 µg mL^{-1}) and efavirenz (120 µg mL^{-1}) measured at 245 nm

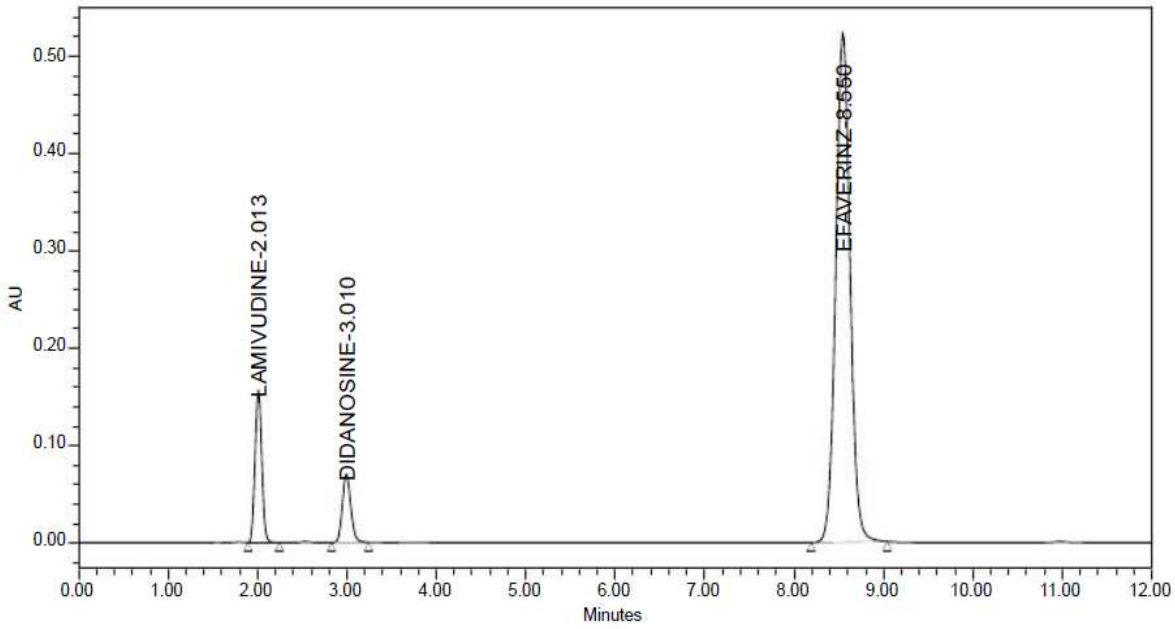


Table 7: Analysis of marketed formulation

Drugs	Labeled amount, mg tablet^{-1}	Amount found, mg tablet^{-1}	% label claim	% *RSD
Lamivudine	300	296.54	98.84	0.095
Didanosine	250	240.42	99.36	0.122
Efavirenz	600	597.64	99.60	0.715

** mean of six observations*

CONCLUSION

RP-HPLC methods enable the quantitation of lamivudine, didanosine and efavirenz in oral dosage form with good accuracy and precision, either in laboratory prepared samples or in pharmaceutical dosage forms. The good recoveries were obtained in all cases as well as the reliable agreement with the reported procedure proved that the proposed methods could be applied efficiently for determination of lamivudine, didanosine and efavirenz in oral dosage form with satisfactory precision. This method is considered simple, reliable, selective providing satisfactory accuracy, precision with lower limits of detection and quantification more specific and sensitive. More over the shorter duration of analysis for lamivudine, didanosine and efavirenz makes the reported method suitable for routine analysis in pharmaceutical dosage forms.

Acknowledgement
Authors are grateful to CIPLA LTD for providing Lamivudine, Didanosine and Efavirenz gift samples and thankful to School of Pharmacy, Anurag group of Institutions, Hyderabad, for providing facilities to carry out research work.

REFERENCES

[1] en.wikepedia.org/wiki/lamivudine accessed on 1-1-**2012**.
[2] http://www.drugbank.ca/drugs/DB00709 accessed on 1-1-**2012**.
[3] en.wikipedia.org/wiki/efavirenz accessed on 1-1-**2012**.
[4] www.drugbank.ca/drugs/DB00900 accessed on 1-1-**2012**.
[5] en.wikipedia.org/wiki/didanosine accessed on 1-1-**2012**.
[6] www.drugbank.ca/drugs/DB00625 accessed on 1-1-**2012**.
[7] G Bahrami; S Mirzaeei; A Kiani; B Mohammadi, *Journal of Chromatography B*, **2005**, 823, 213–217.
[8] AI Veldkampa; RPJ Van Heeswijk; PL Meenhorst; JW Mulder; JMA Lange, JH Beijnen; RMW Hoetelmans, *J. Chromatography B* ,**1999**, 734, 55–61.
[9] CZ Matthews, EJ Woolf a, RS Mazenko, H Haddix-Wiener, CM Chavez-Eng, ML Constanzer, ML Doss; BK Matuszewski, *Journal of pharmaceutical Biomedical Analysis*, **2002**, 28, 925–934.
[10] S Mogatle; I Kanfer, *Journal of Pharmaceutical and Biomedical Analysis*, **2009,** 49 1308–1312.
[11 MS Nacenta; YL Pua; LFL Cortes; J Mallolas; M Jose; X Carne, *Journal of chromatography B*, **2001**, 763, 53–59.
[12] EK Kano; CH Reis Serra; EEM Koono; SS Andrade; V Porta, *International journal of Pharmaceutics*, **2005,** 297; 73–79.
[13] V Proust; K. Toth; A. Hulin; A. M. Taburet; F. Gimenez; E. Singlas, *Journal of Chromatography B*, **2000**, 742; 453–458.
[14] S Notari; C Mancone; T Alonzi; M Tripodi; P Narciso; P Ascenzi, *Journal of Chromatography B*, **2008**, 863, 249–257.
[15] N Kapoor, K Sateesh, P Ramesh, *Journal of pharmaceutical and biomedical analysis*, **2006**, 41, 761–765.
[16] C Marzolini, AT Telenti, B Buclin, J Biollaz, LA Decosterd, *Journal of Chromatography B*, **2000**, 740, 43–58.
[17] B Uslu; SA Ozkan, *Analytica Chimica Acta*, **2002**, 466, 175–185.
[18] DH Shewiyo; E Kaale; C Ugullum; MN Sigonda; PG Risha; B Dejaegher; J Smeyers–Verbeke; Y Vander Heyden, *Journal of Pharmaceutical and Biomedical Analysis*, **2011** 54, 445–450.
[19] S Notari, A Bocedi; G Ippolito; P Narciso, L PaoloPucillo; G Tossini; RP Donnorso; F Gasparrini; P Ascenzi, P, *Journal of Pharmaceutical and Biomedical analysis*, **2006**, 831, 258–266.
[20] A Checa; R Oliver; S Hernandez-Cassou, J Saurina, *Analytica Chimica Acta*, **2008,** 616, 85–94.
[21] ICH, Q2B Validation of Analytical Procedure: Methodology, International Conference on Harmonization, Geneva, March 1996.