Original Article

Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatography (RP-HPLC) method have been developed, which can separate and quantitatively estimate lamivudine and efavirenz in pharmaceutical dosage form. The chromatographic separation for lamivudine and efavirenz was achieved with mobile phase containing 0.1 % triethylamine (pH adjusted to 5.11 with 0.1% orthophosphoric acid) and acetonitrile (30:70 % v/v), reverse phase phenomenex® (Luna 5µ C18(2) 100A (250 × 4.60 mm i.d) column in isocratic mode at room temperature and UV detection at 245 nm. The compounds were eluted at a flow rate of 1.0 ml/min. The retention times of lamivudine and efavirenz were found to be 2.271±0.177 min and 7.267±0.513 min respectively. The above method was validated in terms of linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) etc. in accordance with ICH guide lines. The developed method was free from interferences due to excipients present in tablets. The method was rapid, simple and suitable for routine quality control analysis.

Keywords: - Lamivudine; efavirenz; Method development; Validation; HPLC.

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs that were 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), fusion inhibitors.

Lamivudine ((4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2 dihydropyrimidin-2-one) is a nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus (Fig. 1.). 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]

Efavirenz ((4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one) is a non-nucleoside reverse transcriptase inhibitors. It is used in the treatment of HIV infection (Fig. 2.). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme and therefore, interferes with viral RNA to DNA directed polymerase activities. [3-4]

Literature survey reveals that there are no isocratic RP-HPLC methods for the simultaneous estimation of lamivudine and efavirenz in the bulk drugs and in the formulation. Methods have been reported for the estimation of lamivudine and efavirenz individually as well as in combination with other drugs by UV

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spectrophotometry [5-6] and HPLC [7-14]. To our knowledge, no study related to the isocratic analysis of lamivudine and efavirenz have been reported in literature. Therefore, there is a challenge to develop RP-HPLC method for the simultaneous estimation of lamivudine and efavirenz. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate and precise RP-HPLC method for the simultaneous estimation of lamivudine and efavirenz in pharmaceutical dosage form.

MATERIALS AND METHODS

Acetonitrile (HPLC grade), methanol (HPLC grade) were obtained from Merck specialties private limited, Mumbai, India. Water (HPLC grade) was obtained from Qualigen Fine chemicals, Mumbai, India. Triethylamine (GR grade) was obtained from Merck specialties private limited, Mumbai. Ortho-phosphoric acid (GR grade) was obtained from S.D. Fine Chemicals Ltd, Mumbai, India. Pure drugs of lamivudine and efavirenz were kindly supplied as gratis samples by Hetero Pharmaceutical limited (Hyderabad, India). Odivir-250 Kit manufactured by Cipla Pharmaceutical Limited is used for the analysis. The label claim states that this formulation contains lamivudine (300mg), efavirenz (600mg) per kit. HPLC method development and validation were done on SHIMADZU (Japan) liquid chromatograph equipped with LC-20AD pump, LC 20A UV/Vis detector and Rheodyne 7725i injection with a 20 µL loop. For instrument control, data acquisition and processing, the chromatographic system was interfaced to LC solutions software. Other instruments included are Shimadzu electronic balance BL-220H (SHIMADZU corporation, Japan), Value 1 stage vacuum pump Model: VE115, Fast clean ultrasonic cleaner.

The column used for chromatographic separations was reverse phase phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm i.d). The analytical wave length was set as 245 nm and samples of 20 µL were injected. The chromatographic separations were accomplished using mobile phase consisting of 0.1% triethylamine (pH adjusted to 5.11 with 0.1% orthophosphoric acid) and acetonitrile (30:70 % v/v), filtered through 0.45 µm filter using Value 1 stage vacuum pump and deaerated in fast clean ultrasonic cleaner. Mobile phase was pumped in isocratic mode at a flow rate of 1 ml/min at room temperature.

Preparation of standard solutions

Stock solutions were prepared by dissolving 10 mg of lamivudine, 10 mg of efavirenz in 50 ml of acetonitrile separately. Aliquots of the standard stock solutions of lamivudine and efavirenz were transferred into 100 ml volumetric flasks and solution was made up to the volume to yield required concentrations of lamivudine and efavirenz. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in Fig. 3.

Preparation of sample solution for assay

Twenty Odivir-250 kits each containing 300mg of lamivudine and 600mg of efavirenz were weighed, average weight was calculated and powdered. A quantity equivalent to 300mg of lamivudine and 600mg of efavirenz was weighed and transferred into 100 ml volumetric flask. It was extracted with acetonitrile. 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 acetonitrile and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity range.

RESULTS AND DISCUSSION

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters like symmetry, theoretical plates and capacity factor. Symmetrical peaks with good separation (retention time for lamivudine was 2.271±0.177 min and efavirenz was 7.267±0.513 min) were obtained with reverse phase phenomenex® (Luna 5µ C18(2) 100A (250 × 4.60 mm i.d) column. The mobile phase containing 0.1 % triethylamine (pH adjusted to 5.11 with 0.1% orthophosphoric acid) and acetonitrile (30:70 % v/v) was used at a flow rate of 1.0 ml/min. The optimum wavelength for detection and quantification was at 245 nm, at which good detector response was obtained for both the drugs. There is no interference from the diluents, excipients present in the pharmaceutical formulation.

Method validation:

The proposed method was validated as per International Conference on Harmonization (ICH) guidelines.

Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 40-100 µg/ml for lamivudine, 10-80 µg/ml 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 0.998 and 0.997 for lamivudine and efavirenz respectively (Fig.4 and Fig.5). The results are given in Table 1.

Precision

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done at three levels (intraday, inter day and repeatability). Intraday precision was done by analyzing the intermediate concentration of each drugs (lamivudine 70 µg/ml and efavirenz 40 µg/ml) for three times. Interday precision was measured over three consecutive days for the same drug concentrations. Reproducibility of the method was determined by performing by the same analytical procedure at different laboratories. The %RSD values are calculated for each of them and the low RSD values indicate that the method is precise. The results are given in Table 1.

Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of standard lamivudine and efavirenz corresponding to 50, 100 and 150 % of label claim had been added. At each level of the amount six determinations were performed. The results are given in Table 1.

Sensitivity and selectivity

LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by the equations

given in the ICH guide lines. The LOD with signal/noise ratio of 3:1 were found to be 0.004 µg/ml for lamivudine and 0.02 µg/ml for efavirenz. The LOQ with signal/noise ratio of 10:1 were found to be 0.03 µg/ml for lamivudine and 0.07 µg/ml for efavirenz. The results are given in Table 1.

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.

Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for lamivudine 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.

System suitability studies

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As), tailing factor etc., were studied. The results are given in Table 1.

Analysis of marketed formulation

The proposed procedures were successfully applied for the simultaneous estimation of lamivudine and efavirenz in the formulation and the drug contents in each sample were calculated by comparision with the appropriate standard solution of the drug. The results obtained were in agreement with label claim. The results of analysis are given in Table 2. No interference due to excipients was detected in the chromatograms produced (Fig. 6).

CONCLUSION

RP-HPLC methods enable the quantitation of lamivudine 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, 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 and efavirenz makes the reported method suitable for routine analysis in pharmaceutical dosage forms.

Table 1: Summary of validation parameters

Parameters (units)	Lamivudine	Efavirenz
Linearity range (µg/ml)	40-100	10-80
Correlation coefficient	0.998	0.997
Slope	34207	44252
Intercept	40911	98425
LOD (µg/ml)	0.004	0.02
LOQ (µg/ml)	0.03	0.07
Recovery (%)		
50	99.61	99.66
100	100.05	99.70
150	99.55	99.45
Precision (% RSD)		
Intraday (n=3)	0.301	0.281
Inter day(n=3)	0.548	0.489
Repeatability	0.419	0.297
Theoritical plates	5177	11751
Assymetry factor	1.0	1.33
Tailing factor	0.896	1.132

Table 2: Analysis of marketed formulation

Drugs	Labeled amount, mg/tablet	Amount found, mg/ tablet	% *RSD
Lamivudine	300	298.923	0.095
Efavirenz	600	598.675	0.715

* mean of six observations

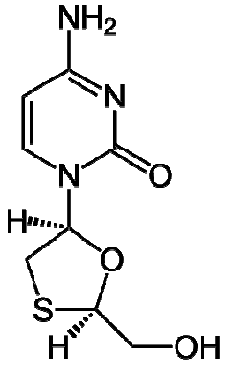

Fig. 1: Structure of lamivudine

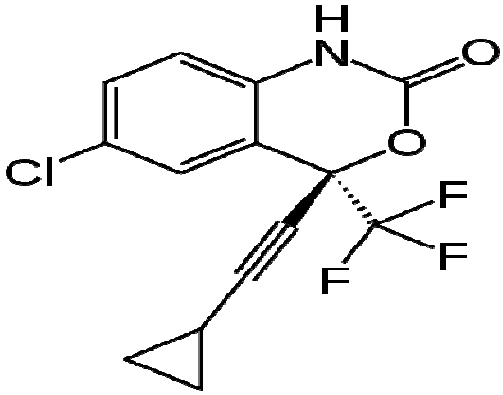

Fig. 2: Structure of efavirenz

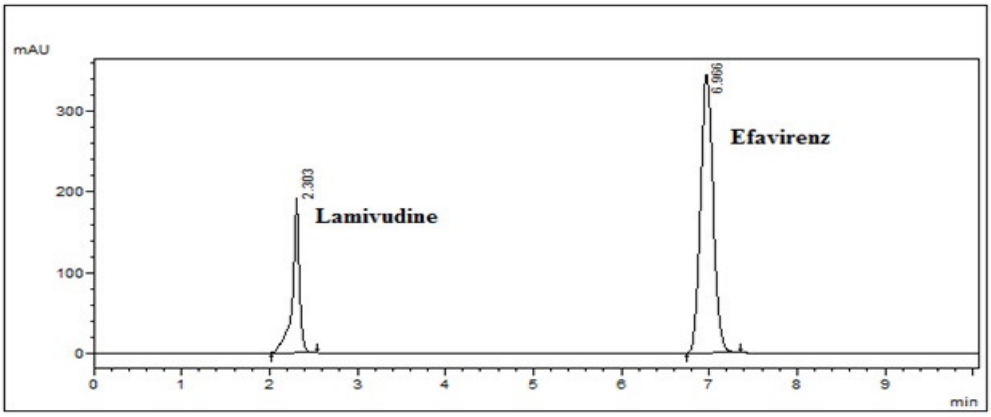

Fig.3: Chromatogram of standard solution (30 µg/ml Lamivudine and 60 µg/ml Efavirenz)

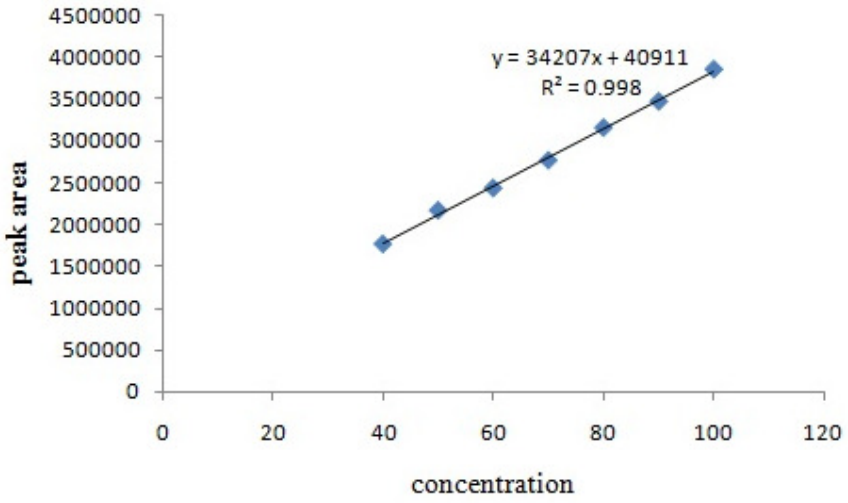

Fig. 4: Calibration Graph of Lamivudine by RP-HPLC

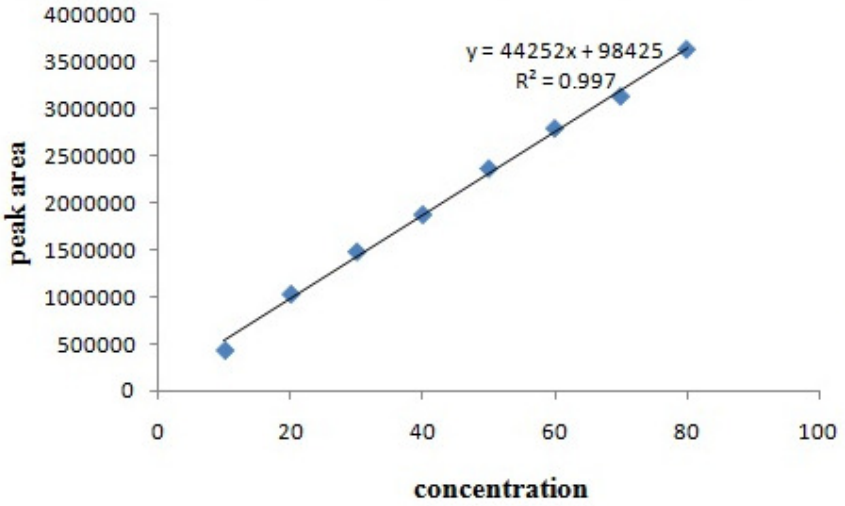

Fig. 5: Calibration Graph of Efavirenz by RP-HPLC

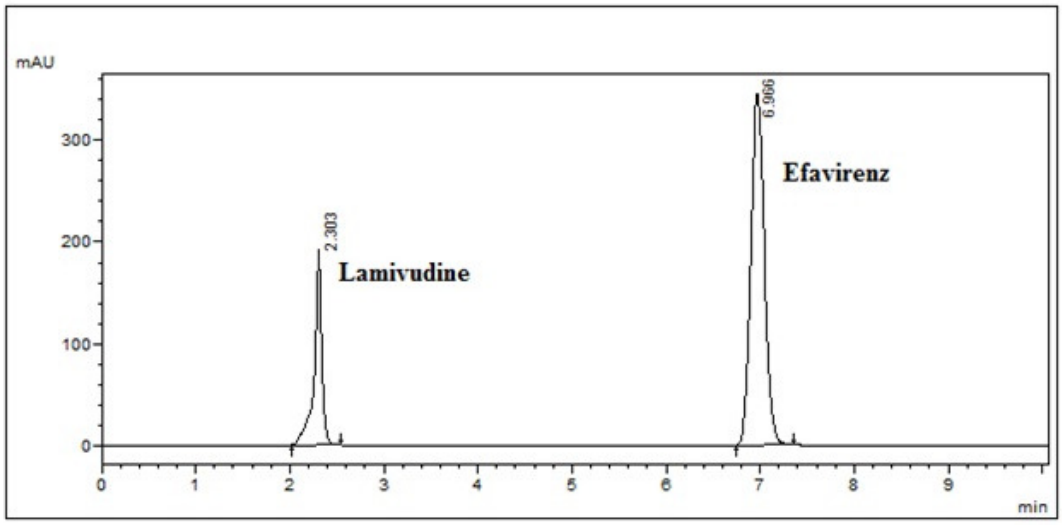

Fig.6 Chromatogram of formulation
(30 µg/ml Lamivudine and 60 µg/ml Efavirenz)

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REFERENCES

1. en.wikepedia.org/wiki/lamivudine accessed on 1-1-2012.
2. 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/DB00625 accessed on 1-1-2012.
5. Deepali G., Elvis, M. UV spectrophotometric method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient and in its tablet formulation. Pharm analysis. 2010; 2 (4):417-419.
6. Anand Kumar Y., Rama Rao N. Development of rapid UV Spectrophotometric method for the estimation of efavirenz in formulations. E-Journal of Chemistry. 2010; 7(3):856-860.
7. Patro S.K., Swain S.R., Patro V.J., Choudhury N.S.K. Development and validation of High performance method for determination of

lamivudine from pharmaceutical preparation. E-Journal of Chemistry. 2010; 7(1):117-122.

8. Kano E. K., Reis Serra C. H., Koono E. E. M., Andrade S. S., Porta V. Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies. Int. J. Pharm. 2005; 297:73–79.
9. Checa A., Oliver R., Cassou SH., Saurina J. Reversed-phase liquid chromatographic method with spectrophotometric detection for the determination of antiretroviral drugs. analytica chimica acta. 2008: 616:85–94.
10. Bahrami G., Mirzaeei.S., Kiani A., Mohammadi B. High-performance liquid chromatographic determination of lamivudine in human serum using liquid–liquid extraction; application to pharmacokinetic studies. J. Chromat. B, 2005; 823:213–217.
11. Veldkamp A.I., Van Heeswijk R.P.J., Meenhorst P.L., Mulder J.W., Lange J.M.A., Beijnen J.H., Hoetelmans, R.M.W. Quantitative determination of efavirenz (DMP 266), a novel non-nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromat. B. 1999; 734:55–61.
12. Matthews C.Z., Woolf E.Z., Mazenko R.S., Haddix wiener H., Chavez Eng C.M., Constanzer M.L., Doss G.A., Matuszewski B.K. Determination of efavirenz, a selective nonnucleoside reverse transcriptase inhibitor, in human plasma using HPLC with post column photochemical derivatization and fluorescence detection. J.Pharm.Biomed.analy. 2002; 28:925–934.
13. Marzolini C., Telenti A., Buclin T., Biollaz J., Decosterd L.A. Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction. J. Chromat. B. 2000; 740:43–58.
14. Proust V., Toth K., Hulin A., Abure A.M., Gimenez F., Singlas E. Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma. J. Chromat. B. 2000; 742:453–458.

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