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Research Paper

ANALYTICAL METHOD VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, TENOFOVIR AND DORAVIRINE

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A simple and new isocratic RP-HPLC method was developed and validated for the estimation ofLAM, TDF and DOR. The chromatographic separation was performed on Waters column (250×4.6mm, 5µm), mobile phase used for the analysis was prepared by the combination of 20.0 parts of Acetonitrile, 30.0 parts of Methanol and 50.0 parts of orthophosphoric acid mixture. The run time for the separation was fixed at 8min and the flow rate was maintained at 1 ml/min with the detection wave length of 260nm. The column temperature was maintained at 25^0C ±5and performed the HPLC analysis. The retention times found to be 2.53 min, 3.53 min and 5.69 min for LAM, TDF and DOR respectively. Under these optimized conditions the respective drugs were shown symmetrical peaks with low tailing factor and high peak area without interference of any excipients.

Keywords: Lamivudine, Tenofovir, Doravirine, RP-HPLC and Validation.

INTRODUCTION

Any product or service needs analysis, but since drugs involve human life, they require it much more. The study of separation, measurement, and identification of chemical additives is known as analytical chemistry.[1]

Lamivudine is a synthetic nucleoside analogue used in the treatment of HIV infection and chronic hepatitis B. It belongs to the class of nucleoside reverse transcriptase inhibitors (NRTIs) and is commonly used in combination with other antiretroviral drugs.[2]

Lamivudine is a cytidine analogue that requires intracellular phosphorylation to its active triphosphate form. The active metabolite competitively inhibits viral reverse transcriptase and is incorporated into viral DNA, causing chain termination due to the absence of a 3'-OH group. This inhibits viral DNA synthesis and prevents replication of HIV and hepatitis B virus. Tenofovir is a nucleotide analogue antiretroviral drug used in the treatment of HIV-1 infection and chronic hepatitis B. It belongs to the class of nucleotide reverse transcriptase inhibitors (NtRTIs) and is commonly used in combination with other antiretroviral agents.[3]

Tenofovir is an adenosine monophosphate analogue administered as oral prodrugs such as Tenofovir disoproxilfumarate and Tenofovir alafenamide. After absorption, it is converted intracellularly to tenofovir diphosphate, the active metabolite. This metabolite competitively inhibits viral reverse transcriptase and is incorporated into viral DNA, leading to chain termination and inhibition

of viral replication.[4-5]

Doravirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in the treatment of HIV-1 infection. It is administered orally and is usually given in combination with other antiretroviral agents. Doravirine selectively inhibits HIV-1 reverse transcriptase by binding to an allosteric (non-active) site on the enzyme, producing a conformational change that blocks viral RNA-dependent DNA polymerization and prevents viral replication.[6-7]

Chemically, doravirine is a diaryl-pyridinone derivative containing halogen-substituted phenyl and trifluoromethyl groups that enhance lipophilicity and binding to the NNRTI pocket. Unlike nucleoside analogues, it does not require intracellular phosphorylation for activity and acts as a non-competitive inhibitor.

This is a new combination is market and so far no suitable analytical methods have been reported for simultaneous analysis of both the drugs together.

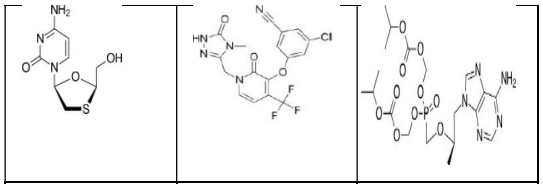

Fig. 1: Chemical Structure of (A) Lamivudine (B) Doravirine (C) Tenofovir[8-9]

Materials Used:

Lamivudine, Tenofovir Disoproxil Fumerate, & Doravirine – M/s Hetero Pharma, India

Chemicals and Reagents:

- Acetonitrile (ACN)- HPLC grade and AR grade
- Methyl alcohol (MeOH) - HPLC grade and AR grade
- Ortho phosphoric acid (OPA) HPLC grade

Instrument

Analytical weighing balance made by Mettler. The digital pH meter made by Labtronic Laboratory. HPLC by Shimadzu class VP with software from M/s LC Solutions.

Chromatographic conditions

Mobile Phase : OPA: MeOH: ACN (50:30:20)

Column : Waters, C18, 250X4.6mm, 5µm

Flow Rate : 1.0mL/Min

Sample Temperature: 25^{0}C

Column Temperature: 25^{0}C

Volume : 10µL

Run time : 8 min

Detector : 260

pH : 4.5

Standard solution

300 mg LAM, 300 mg TDF, and 100 mg DOR were carefully weighed in a flask, and milliliter of mobile phase was mixed. The solution was agitated for 20 min with occasional stirring for optimal drug dissolution. To the solution mobile phase was added, filtered and allowed to cool

down. In a flask, 1 mL of the prepared solution is mixed into mobile phase.

Sample solution

10 powdered samples were taken and combined to yield a total weight of 776.00mg (300 mg LAM, 300 mg TDF, and 100 mg DOR); it was dissolved in 50 mL of mobile phase in flask. Sonication was performed for 20 min with occasional stirring to eliminate all of the drugs. Before using, the solution was diluted using diluant, strained, and allowed to cool at ambient temperature. 1 ml of the prepared solution was mixed with mobile phase, and diluted once more. The three drugs provided were LAM (300 mg), TDF (300 mg), and DOR (100 mg).

Method validation

The proposed method was validated as per the ICH guideline Q2(R1).

RESULTS AND DISCUSSION:

System Suitability

System suitability can be measured by observing the peaks obtained from different chromatograms of drug samples. For drug samples, 6successive feeds of the standard solution demonstrated equal RT, TP, TF, &Rs, indicating a satisfactory analytical apparatus.

Specificity

Specificity is the potential that judges the presence of impurities in the analyte. It can be determined by comparing the chromatograms obtained for blank and drug solutions.

Blank, standard and sample solutions were fed into HPLC column as per the test procedure and chromatograms were noted.

RTs for LAM, TDF & DOR are the identical for the standard, test samples as shown in the chromatograms. It verifies the approach since additives do not influence the analytical technique. However, no overlap was observed between the blank and drug peaks. As a result, it is a highly efficient means of method selection.

Linearity

Peak areas and concentrations of 50 percent through 150 percent of the actual concentration were found to be linearly related to LAM, TDF & DOR. The correlation coefficients of LAM, TDF & DOR were reported to be 1.000, 1.000, and 0.999,accordingly, indicating that the technique is linear across 50 percent to 150 percent.

LOD and LOQ

LOD is the smallest analyte concentration that can be sensed, but not always measured, is known as the limit of detection. LOD values for LAM, TDF & DOR were reported to be 0.7746, 0.740 & 0.769, accordingly

LOQ is the smallest analyte concentration at which peak can be observed & reliably quantified is the limit of quantitation. LOQ values for LAM, TDF & DOR were reported tobe 2.5820, 2.467, & 2.565, accordingly.

Accuracy

The reported value was obtained during a recovery test. Quantity of every drug

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Table 1: Specificity data for Lamivudine, Tenofovirdisoproxilfumarate and Doravirine

S. No	Sample	Lamivudine area	RT	Tenofovir disoproxil fumarate area	RT	Doravirine Area	RT
1	Standard	2623699	2.531	3511298	3.530	1690765	5.691
2	Sample	2601410	2.526	3489831	3.516	1679238	5.653
3	Blank	-	-			-	-
4	Placebo	-	-			-	-

administered was evaluated against the quantity retrieved. Recovery rates for LAM, TDF & DOR were 100%, 100%, and 101%, accordingly. The technique's precision was proved several times.

Precision

The developed method is repeatable and precise, as the %RSD value for the assay was precise, as the %RSD value for the assay was below 2.0 demonstrates the method repeatability and intermediate precision.

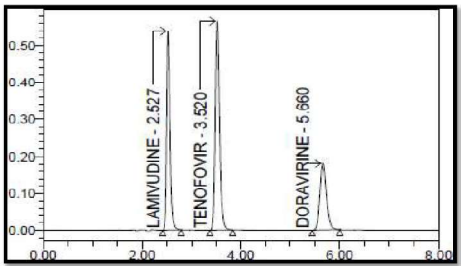

Fig. 2 A: Chromatogram of Standard

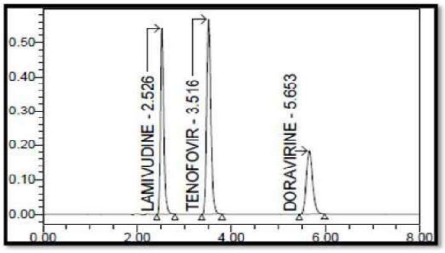

Fig. 2 B: Chromatogram of Sample

CONCLUSION:

This method was validated on the report of precision, accuracy, specificity, linearity. In all the cases the method was stable with acceptance criteria followed by ICH guidelines. Mobile phase used in this method was very commonly available and it is sufficient for the quantification analysis of Lamivudine, Tenofovir and Doravairine either in single dosage or in combination form.

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Conflict of Interest

The authors declare that they have no conflict of interest