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Stability-Indicating HPTLC Method development and validation for Analysis of Lamivudine and Tenofovir from its Tablet Dosage Form

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ABSTRACT:

A simple and sensitive thin-layer chromatographic method has been established for analysis of Lamivudine (LAM) and Tenofovir (TEN) in pharmaceutical dosage form. Chromatography on silica gel 60 F_{254} plates with Chloroform and methanol in the ratio of 9:1 (v/v) as a mobile phase furnished compact spots at Rf 0.06±0.01 for LAM and 0.33±0.01 for TEN. Densitometric analysis was performed at 254 nm. Both the drugs were subjected to acid, base, neutral hydrolysis, oxidation, photolysis, and thermal decomposition, and the peaks of degradation products were well resolved from that of the pure drug. The maximum decomposition was detected with the acid, base, and oxidation catalyzed degradation. Linear regression analysis revealed a good linear relationship between peak area and amount of LAM and TEN in the concentration range of 100–700 ng/band respectively. Limit of detection was found 33.06/spot and 33.20 ng/spot for LAM and TEN respectively. Limit of quantification was found 100ng/spot for LAM and 110ng/spot for TEN. The method was validated, in accordance with ICH guidelines for precision, accuracy, and robustness. Since the method could effectively separate the drug from its degradation products, it can be regarded as stability indicating.

KEYWORDS: HPTLC, Stability Indicating Method, Lamivudine, Tenofovir.

Introduction:

Lamivudine Chemically it is (2R, 5S)-4-Amino [2-(Hydroxy methyl)-1, 3-oxathiolan-5yl]-2(1H) –Pyrimidinedione (Fig No.1). It is used in HIV infection.Lamivudine is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of

action of 3TC-TP inhibition of RT via DNA chain termination after in corporation of the nucleotide analogue.

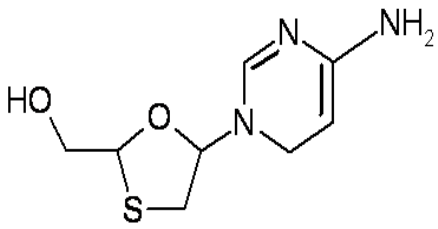

Fig No. 1: Chemical Structure of Lamivudine

Tenofovir Chemically it isBis (hydroxymethyl) [[(R)-2(6-Amino- 9H-purin-9-yl)-1-methylethoxy] methyl]phosphonate,bis(isopropylcarbonate)(ester)fumarate(1:1)(Fig No.2). It is nucleotide analogue reverse transcriptase inhibitors (NtRTIs). It is official in Indian Pharmacopoeia 2007.

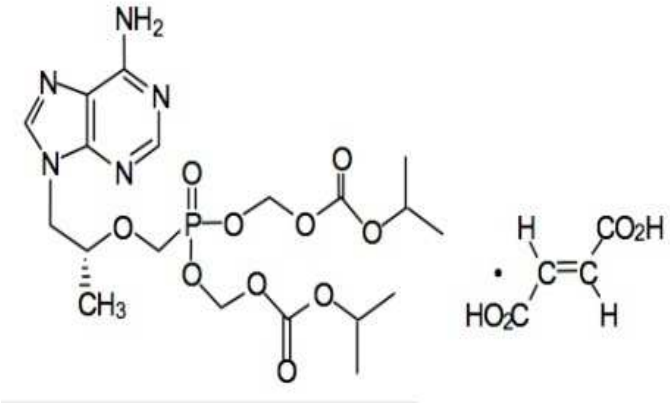

Fig No. 2: Chemical Structure of Tenofovir

Both the drugs are marketed as combined tablet dosageformulation in the Ratio is 300:300mg LAM &TEN.Literature Survey revealed that a number of Method havebeen reported for estimation of both the drugs individually and in combination of other drugs, but not a single method is reported for simultaneous estimation of LAM &TEN by stability indicating HPTLC.So present work describes stability indicating method for simultaneousestimation of LAM and TEN in tablet formulation.

Experimental:

Materials and Reagents

LAM and TEN were obtained from Cipla Pharmaceuticals Ltd (India). It was used without further purification and certified to contain 99.67% and 99.83% (w/w)respectively on dry weight basis. LAM and TEN Pharmaceutical preparation, TENVIR – L was purchased from a local drug store; it contained 300 mg of each drug substance. The other chemicals and reagents used were of AR grade and procured from S.D. Fine-Chem (New Delhi, India).

Preparation of developing solvent (mobile phase):

Developing solvents consisting of more than one component are prepared by measuring the required volume of chloroform (9 ml) , methanol (1ml),and transferred into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content.

HPTLC Instrumentation

Chromatography was performed on 20 cm × 10 cm aluminium foil plates precoated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Germany). Before use, the plates were prewashed by development with methanol then dried in the current of dry air and activated at 60°C for 5 min. Samples were applied as bands 6 mm wide, 15 mm apart, by use of a Camag (Switzerland) Linomat 5 equipped with a microlitre syringe. A constant application rate of 150 nL s^{-1} was used. Chloroform and methanol 9 : 1 (v/v) was used as the mobile phase. Linear ascending development was performed in a twin-trough glass chamber previously saturated with mobile phase vapour for 30 min at room temperature (RT, 25 ± 2°C) and relative humidity 60 ± 5%. The development distance was approximately 80 mm. After development, the plates were dried in current of air by use of an air dryer. Densitometric scanning, at 254 nm, was performed with a Camag TLC scanner 3 in absorbance mode. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm. UV spectrum is recoded as in Fig. No. 3

Analysis of LAM and TEN in Tablet Dosage Form

To determine the LAM and TEN content of conventional tablets, twenty tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 3 mg of both LAM and TEN were transferred to a 100 mL volumetric flask, extracted with methanol, sonicated for 20 min, and diluted to volume with same solvent. The resulting solution was filtered through a 0.45 μm filter (Millifilter; Milford, MA; USA). The solution (3 μL, 300 ng of LAM and TEN each) was applied in triplicate on an HPTLC plate for quantification using the proposed method. (Fig. No. 4 and 5)

Method Validation

Linearity of Lamivudine and Tenofovir

A stock solution containing 100 μg/mL of LAM and TEN respective was prepared by dissolving an accurately weighed 10 mg portion of each drug in methanol in separate 100 mL volumetric flasks. Different volumes of stock solution 100 – 700 ng/band were spotted on an HPTLC plate of both the drugs in triplicate. The data of peak area versus drug concentration were treated by linear least-squares regression. (Fig. No. 6)

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyzing three different concentrations (100, 200, and 300 ng/spot) of the drug by six times on the same day. The intermediate precision of the method was checked by repeating on three different days. (Table. No. 1)

Robustness

The analytical conditions were deliberately changed, by introducing small changes in mobile phase composition (±2%), mobile phase volume (±2%), chamber saturation period (±10%), and development distance (±10%), and time from application to development (0, 10, 15, 20 min) and time from development to scanning (0, 30, 60, 90 min) were carried out.

LOD and LOQ

The method was used to determine the LOD and LOQ. Blank methanol was spotted six times, and the SD of the peak area of the blanks was calculated. The limits were determined from the slope of the calibration plot and the SD of the response for the blank sample by use of formula.

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80, 100, and 120%. Known amount of standard of LAM and TEN were added to pre-analysed samples and was subjected to the proposed HPTLC method.(Table. No. 1)

Specificity

The specificity of the method was determined by comparing the results for the standard drug and the sample. The peak purity of the sample was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the band.

Forced Degradation Studies of Lamivudine and Tenofovir

Acid Induced Degradation

HCL (0.1 N, 10ml) was added separately to 10 mL methanolic stock solution of LAM and TEN and mixture in 10 mL volumetric flasks. Solution was reflux at 80^0C for 2 Hrs, neutralized the solution with 0.1 N NaOH, 1000ng/spot of each drugis spotted on plate.(Fig. No. 7) (Table. No. 2)

Base Induced Degradation

NaOH (0.1 N, 10ml) was added separately to 10 mL methanolic stock solution of LAM and TEN and mixture in 10 mL volumetric flasks. Solution was reflux at 80^0C for 2 Hrs, neutralized the solution with 0.1 N HCL, 1000 ng/spot of each drugis spotted on plate.(Fig. No. 8) (Table. No. 2)

Hydrogen Peroxide Induced Degradation

H_2O_2 (30 %, 10 mL) was added separately to 10 mL methanolic stock solution of LAM and TEN and mixture in 10 mLvolumetric flasks. Solution was kept for 2 Hr, 1000 ng/spot of each drugis spotted on plate.(Fig. No. 9) (Table. No. 2)

Photo stability Studies

Photodegradation was performed by spreading the drug substance in Petri dish as thin film and kept in photo stability chamber equipped with ultraviolet light with energy of not less than 200 Watt hours/Square meter and fluorescence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved and diluted to get 1000 ng/spot of each drugis spotted on plate.

Dry Heat Studies

Susceptibility to dry heat was studied by exposing the solid drugs to 60ºC for 3 days. The samples were withdrawn at appropriate times, diluted appropriately to get 1000 ng/spot of each drugis spotted on plate.

Result and Discussion

The mobile phase consisting of chloroform: methanol (9: 1 v/v) and the detection spot was carried out at 254 nm and gave R_f values of 0.06±0.01 and 0.33±0.01 for LAM and TEN, respectively with dense and compact spots desired for quantification of LAM and TEN in pharmaceutical formulations. Calibration curve was obtained in range 100 – 700 ng / ml with correlation coefficient values of 0.9990 and 0.9996 for LAM and TEN respectively. Assay of tablets was 99.16% and 99.94 for LAM and TEN respectively. Recovery was found to be 99.54% for LAM and 99.69% for TEN. Limit of detection was found 33.06/spot and 33.20 ng/spot for LAM and TEN respectively. Limit of quantification was found 100ng/spot for LAM and 100ng/spot for TEN. Stability testing was carried out by use of 0.1 N HCl, 0.1 N NaOH, 30% hydrogen peroxide, thermal degradation and photo light degradation.Both the drugs were prepared with a concentration of 1000 µg/ mL and then kept for respective time interval. During analysis it was found that LAM and TEN shows degradation pattern in acid, alkaline, and

oxidation. Hence, the proposed method is stability indicating and can be successively used to determine the drug content of marketed formulation.

Conclusion:-

The stability indicating HPTLC method developed is simple, precise, specific, rapid and accurate. The statistical analysis proved that method is reproducible and selective for the analysis of LAM and TEN in bulk drug and tablet formulations. The developed methods can be conveniently used by quality control department to determine the assay of pharmaceutical preparation and stability studies.

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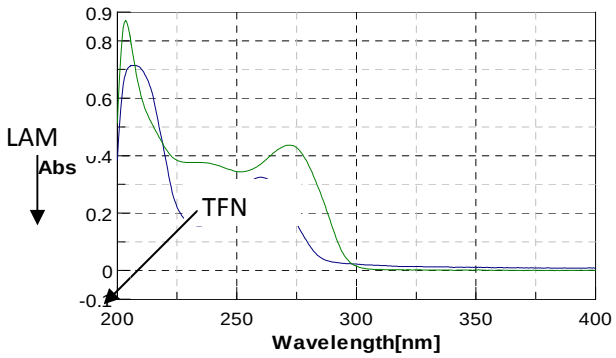

Fig. No. 3 : Overlain spectrum of both LAM and TEN

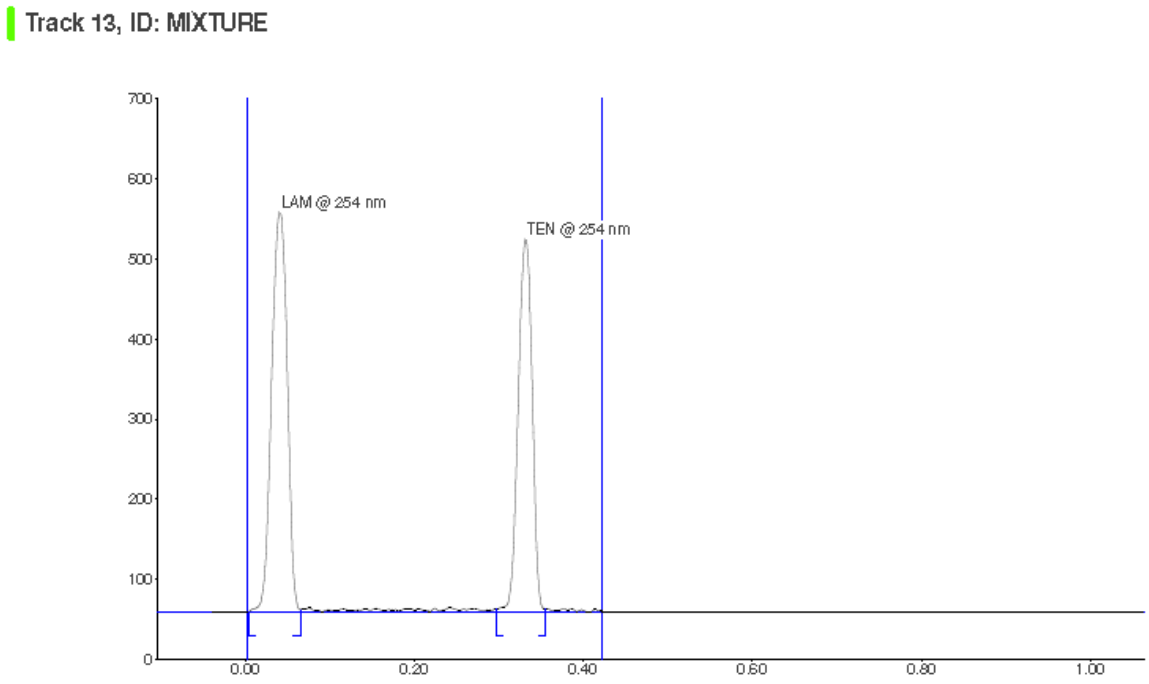

Fig. No.4: Typical HPTLC Chromatogram of LAM &TEN in Tablet Analysis

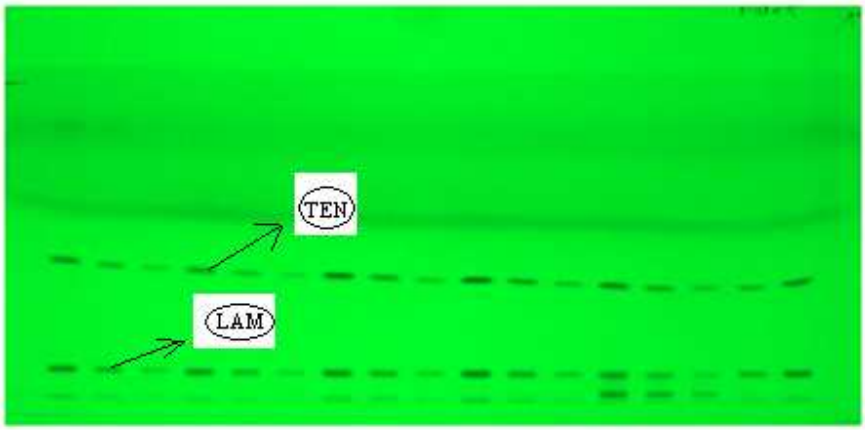

Fig. No. 5 : HPTLC Plate of LAM &TEN in Tablet Analysis

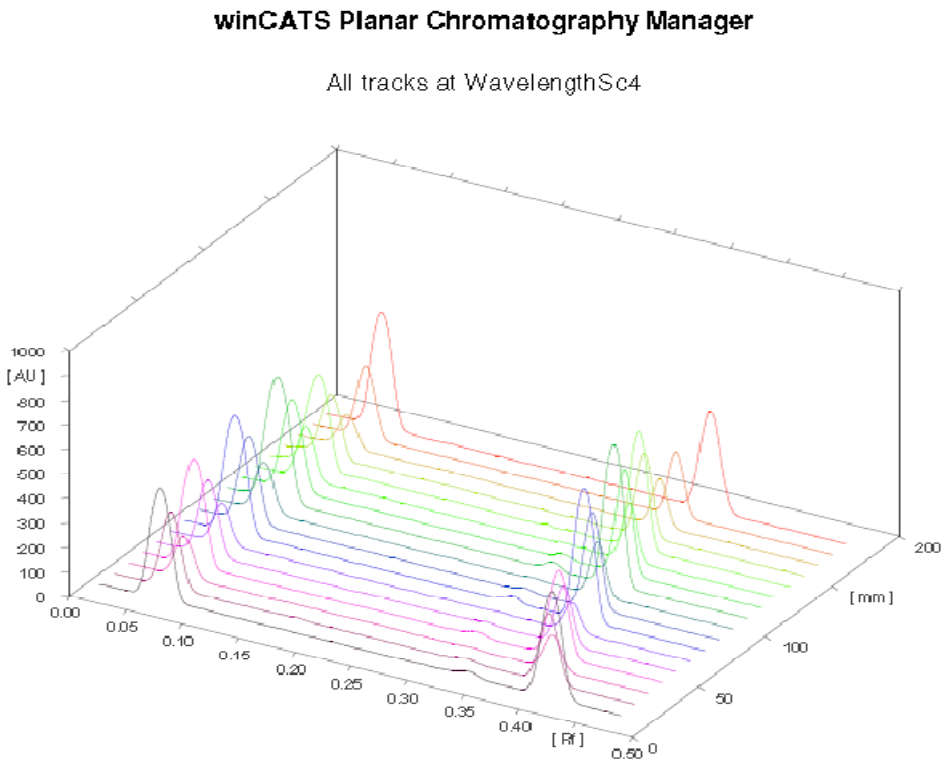

Fig. No. 6: Calibration of LAM and TEN in WinCats Software

Table. No. 1: Summary of Method Validation of LAM and TEN analysis

Parameter	LAM	TEN
Linearity range(ng/spot)	100-700	100-700
Slope	10.651	10.549
Intercept	555.27	523.89
Regression Coefficient	0.358	0.546
Limit of Detection	33.06ng/spot	33.24ng/spot
Limit of Quantitation	100ng/spot	110ng/spot
Precision (%) Interday Intraday	 99.54 99.75	 99.69 99.23
Accuracy 80 100 120	 99.02 99.15 99.59	 99.98 100.56 99.19
Specificity	Specific	

TABLE NO 2: Stability study data of LAM and TEN

Stress Degradation Studies	Max Rf		Percent Degradations (%)		Percent Recovery	
	LAM	TEN	LAM	TEN	LAM	TEN
0.1N HCl refluxed at 80°C for 2 hours	0.23	0.31	7	9	92.32	90.54
0.1N NaOH refluxed at 80°C for 2 hours	0.32	0.36	11	13	89.45	87.85

Neutral hydrolysis (Reflux at 50^o C)	**NA**	**NA**	No degradation	No degradation	100.02	100.14
Oxidation (30% H_2O_2)	0.29	0.31	15	14	85.98	86.23
Thermal degradation	**NA**	**NA**	No degradation	No degradation	99.87	99.56
Photodegradation	**NA**	**NA**	No degradation	No degradation	99.36	99.69

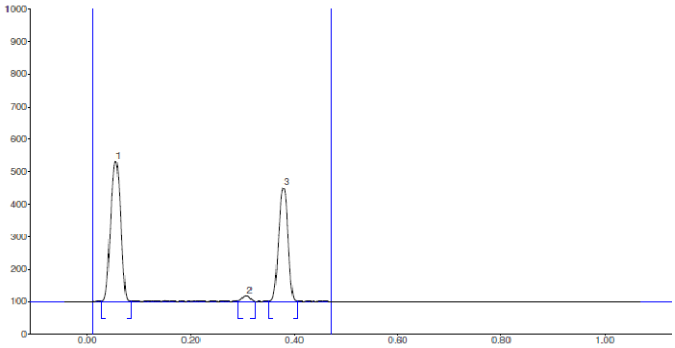

FIGURE NO. 7: TYPICAL CHROMATOGRAM OF LAM and TEN SHOWING ACIDIC HYDROLYSIS.

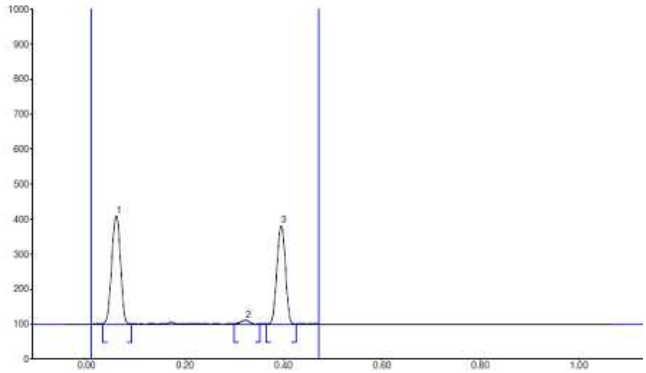

FIGURE NO. 8 : TYPICAL CHROMATOGRAM OF LAMIVUDINE AND TENOFOVIR SHOWING ALKALINE HYDROLYSIS

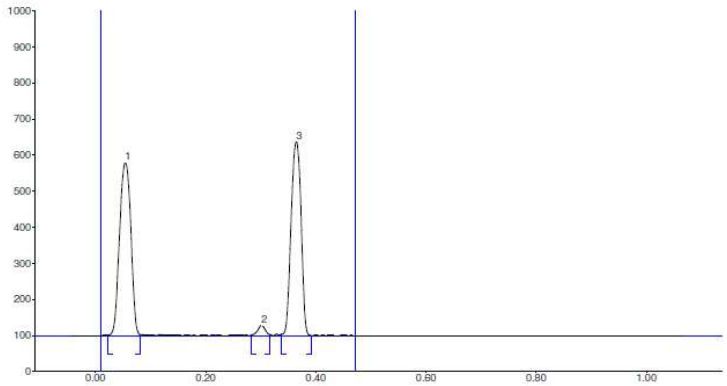

FIGURE NO. 9 : TYPICAL CHROMATOGRAM OF LAMIVUDINE AND TENOFOVIR SHOWING OXIDATION DEGRADATION