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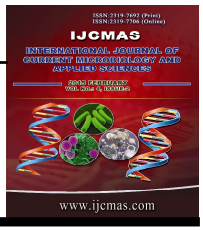

Original Research Article

Determination of Lamivudine and Tenofovir in Pharmaceutical dosage form by Area under Curve and Multicomponant UV Spectrophotometric method

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Keywords

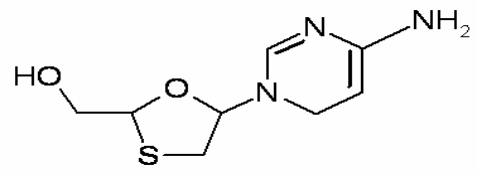
Lamivudine, Tenofovir, UV-Spectro-photometry, Area under Curve, Multicomponant method

A B S T R A C T

Two simple, accurate and precise UV methods were developed for the estimation of Lamivudine (LAM) and Tenofovir (TEN) in Tablet dosage form. Both the drugs are used against HIV infection. Method A is Area under Curve Spectrophotometry and in this the wavelength range selected for Quantitation are 250-280 nm for Lamivudine and 249-269 nm for Tenofovir. Method B is Multicomponant mode wavelength selected for Quantitation method were 271.0 nm (λmax of LAM) and 260.0 nm (λmax of TEN) for the analysis. In both the methods linearity for detector response was observed in the concentration range of 10-60 microgram/ml for TEN and LAM respectively. The results of tablet analysis for Area under Curve was found to be 99.81 ± 0.151 for LAM and 99.50 ± 0.220 for TEN and results obtained for Multicomponant was 99.73 ± 0.306 for LAM and 99.30 ± 0.224 for TEN.. The proposed methods were successfully applied for the Simultaneous determination of both the drugs in bulk as well as commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies which were according to ICH guidelines.

Introduction

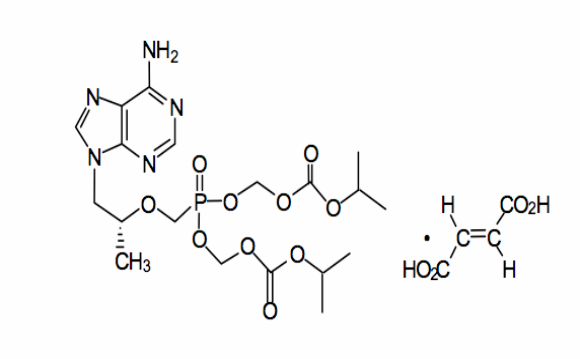
Lamivudine Chemically it is (2R, 5S)-4-Amino1 [2-(Hydroxy methyl)-1, 3-oxathiolan-5yl]-2(1H)-Pyrimidinedione. It is used in HIV infection. The principal mode of action of 3TC-TP inhibition of RT via DNA chain termination after incorporation of the nucleotide analogue.



LAMIVUDINE

Tenofovir is 1-(6-aminopurin-9-yl) propan-2-yl-oxymethyl phosphonic acid.

It is nucleotide analogue Reverse Transcriptase inhibitor (NRTI).



Tenofovir Disproxil Fumarate

Both Tenofovir and Lamivudine are official in IP and are marketed as combined tablet dosage formulation in the Ratio is 300:300 mg LAM: TEN and were named as TENVIR-L as brand name which was produced by Cipla Ltd.

Literature survey revealed that a number of methods have been reported for estimation of both the drugs individually and in combination of other drugs, but not a single method is reported for the estimation of both the drugs simultaneously. Present work describes two precise, accurate, and reproducible methods for simultaneous estimation of TEN and LAM in tablet formulation.

Materials and Methods

Instruments: UV-Visible Spectrophotometer (Double Beam)

Make: Jasco

Model: UV V-630 Spectrophotometer
Spectral Bandwidth: 2nm

Materials: Standard gift sample of Tenofovir and Lamivudine were provided by Cipla Ltd and Combined dose Tablet (Tenvir-L: 300 mg Lamivudine and 300 mg Tenofovir; manufactured by Cipla Pvt. Ltd.), were purchased from local market for analysis.

Solvent used: Double distilled water used as solvent.

Stock solution: Stock solution of both the drugs 300mcg/ml is prepared by dissolving 30mg each drug in 100ml volumetric flask and the final volume is make up by Double distilled water.

Method A – Area under Curve (AUC) Method:

In this method, the stock solution of both the drugs 100mcg/ml is prepared by dissolving 30mg each drug in 100ml volumetric flask and the volume is makeup by Double distilled water, by appropriate dilution of standard stock solutions of both the drugs to 30mcg/ml dilution respectively is scanned in the spectrum mode from 400 nm to 200 nm.

The absorption spectra thus obtained is selected for analysis, from the overlain spectra of both the drugs (fig.3), wavelength selected for Quantitation are 250-280 nm for Lamivudine and 249-269 nm for Tenofovir. The calibration curves for TEN and LAM was plotted in the concentration range of 10-60 mcg/ml exhibiting the Beer's and Lamberts range (table 1, 2). The concentration of individual drug present in the mixture was determined by using the simultaneous AUC equation calculations.

Determination of 'X' values:

$$X = \frac{\text{AUC of component between selected wavelength range}}{\text{Concentration of that component in mgm/lit}}$$

Equation used for determination of concentrations of LAM and TEN

$$C_{LAM} = \frac{X^{TEN}_{250\text{-}280} \times AUC_{249-269} - X^{TEN}_{249-269} \times AUC_{250\text{-}280}}{X^{TEN}_{250\text{-}280} \times X^{LAM}_{249-269} - X^{TEN}_{249-269} \times X^{LAM}_{250\text{-}280}}$$

$$C_{TEN} = \frac{X^{LAM}_{249-269} \times AUC_{250\text{-}280} - X^{LAM}_{250\text{-}280} \times AUC_{249-269}}{X^{TEN}_{250\text{-}280} \times X^{LAM}_{249-269} - X^{TEN}_{249-269} \times X^{LAM}_{250\text{-}280}}$$

Where AUC 269 – 249 and AUC290 – 270 are the area under curves of solution at wavelength range between 249 – 269 nm (TEN) and 250 – 280 nm (LAM) respectively.

Procedure for analysis of tablet formulation

Twenty tablets of Lamivudine and TEN in combination were weighed; their average weight was determined and finally crushed to powder sample. From the triturate, tablet powder equivalent to 30mg of LAM and 30mg of TEN was weighed and transferred to 100ml volumetric flask and dissolved in 50ml Double Distill water and Finally the volume was made upto the mark with Double Distill water. The solution is subjected to ultrasonification for 30min and then filtered through Whatman filter paper No.41.This tablet solution was further diluted to obtain 30µg/ml of LAM and 30µg/ml of TEN respectively. The mixed sample solutions were analyzed to obtain spectra's and the AUC is recorded using wavelength range from 250-280 nm for LAM and 249-269 nm for TEN were noted.

Method B – Multicomponant Method

In this method, the stock solution of both the drugs 100mcg/ml is prepared by dissolving 30mg each drug in 100ml volumetric flask and the volume is makeup by Double distilled water, by appropriate dilution of standard stock solutions of both the drugs to 30mcg/ml dilution respectively is scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained is selected for analysis, from the overlain spectra of both the drugs (fig.4), wavelength selected for wavelength selected for Quantitation are 260.0 nm and 271.0 nm for Tenofovir (TEN) and Lamivudine (LAM) respectively which are the λmax of both the drugs. The calibration curves for TEN and LAM was plotted in the concentration range of 10-60 mcg/ml exhibiting the Beer's and Lamberts range (table 1, 2). The concentration of individual drug present in the mixture was determined by using the simultaneous Multicomponant equation calculations.

Analysis of tablet formulation

Twenty tablets of LAM and TEN in combination were weighed; their average weight was determined and finally crushed to powder sample. from the triturate, tablet powder equivalent to 300mg of LAM and 300 mg of TEN was weighed and transferred to 100 ml volumetric flask and dissolve in 50 ml Double Distill water and the content was kept in ultrasonicator for 30 min. finally the volume was made up to the mark with Double Distill water The solution was filtered through Whatman filter paper No.41.This tablet solution was further diluted to obtain 30 mcg/ml of LAM and 30 mcg/ml of TEN. The mixed sample solutions were analyzed to Multicomponant mode of an instrument.

Result and Discussion

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of LAM and TEN. In AUC method wavelength selected for Quantitation were 250- 280 nm for LAM and 249-269 nm for TEN. In both the methods linearity for detector response was observed in the concentration range of 10-60 mcg/ml for LAM and TEN, both. Percent label claim for LAM in tablet analysis by both the methods was found in the range of 99.81 ± 0.151 for LAM and 99.50 ± 0.220 for TEN. Standard deviation and coefficient of variance for three determination of tablet sample, by both the methods was found to be less than + 2.0 indicating precision of both the methods. Percent recovery was found in the range of 99.98 for LAM and 99.36 for TEN, values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods.

In method B which was Multicomponant method wavelength selected for Quantitation method were 271.0 nm (λmax of LAM) and 260.0 nm (λmax of TEN) for the analysis. Percent label claim for LAM in tablet analysis by both the methods was found in the range of 99.73 ± 0.306 for LAM and 99.30 ± 0.224 for TEN. Standard deviation and coefficient of variance for three determination of tablet sample, by both the methods was found to be less than + 2.0 indicating precision of both the methods.

Table.1 X value for LAM and TEN

Components	X Value at	
	250-280 nm	**249 – 269 nm**
LAM	362.63	339.85
TEN	183.31	438.81

Table.2 Analysis of LAM and TEN in Table

Component	Label Claim (mg/tablet)	Drug Content (%) ± SD	± SEM
LAM	300	99.81 ± 0.1513	**0.0676**
TEN	300	99.50 ± 0.2240	0.1001

*n=3

Table.3 Statistical Parameters

Component	Mean	Standard Deviation	Co-efficient of Variation	Standard Error
LAM	99.81 %	0.1513	0.151302	0.0676
TEN	99.50 %	0.2240	0.2240028	0.1001

Table.4 Accuracy study Data of LAM and TEN by AUC method

Level of % Recovery	Amount present (mg/tab)		Amount of standard added (mg)		Total amount recovered (mg)		%Recovery	
	LAM	TEN	LAM	TEN	LAM	TEN	LAM	TEN
80	300	300	240	240	539.88	536.05	99.98	99.27
100	300	300	300	300	599.80	595.02	99.97	99.47
120	300	300	360	360	659.78	665.11	99.97	99.36

*n=3

Table.5 Statistical Parameters

Component	Mean	Standard Deviation	Co-efficient of Variation	Standard Error
LAM	99.98	0.015	0.0150	0.1
TEN	99.36 %	0.055	0.0550	0.1

Table.6 Analysis of LAM and TEN in Tablet

Component	Label Claim (mg/tablet)	Drug Content (%) ± SD	± SEM
LAM	300	99.73 ± 0.306	**0.1369**
TEN	300	99.30 ± 0.224	0.1001

Table.7 Statistical Parameters

Component	Mean	Standard Deviation	Coefficient of Variation	Standard Error
LAM	99.73%	0.313	0.3135	0.128
TEN	99.30 %	0.224	0.2255	0.100

Table.8 Accuracy study Data of LAM and TEN by Multicomponant Method.

Level of % Recovery	Amount present(mg/tab)		Amount of standard added(mg/tab)		Total amount recovered(mg)		% Recovery*	
	LAM	TEN	LAM	TEN	LAM	TEN	LAM	TEN
80	300	300	240	240	539.64	536.05	99.95	99.27
100	300	300	300	300	599.8	595.02	99.97	99.17
120	300	300	360	360	660.2	665.11	100.02	99.26

Table.9 Statistical Parameters of LAM and TEN by Multicomponant Method

Component	Mean	Standard Deviation	Coefficient of Variation	Standard Error
LAM	99.98%	0.036	0.0361	0.0208
TEN	99.23 %	0.0552	0.0555	0.0317

Table.10 Validation Parameters of LAM and TEN

Method characteristics	Lamivudine	Tenofovir
Linearity	5-60 µg/mL	5-60 µg/mL
Regressions equation	y = 0.0175 + 0.0038	y = 0.0239 + 0.0057
Correlation coefficient	0.999	0.999
LOD(µg/mL)	0.239	0.472
LOQ(µg/mL)	0.791	1.56
Precision (RSD, %)		
Intraday (n=3)	0.330	1.182
Interday (n=3)	0.556	0.759
Specificity	Specific	Specific

Fig.1 Calibration Curve of LAM

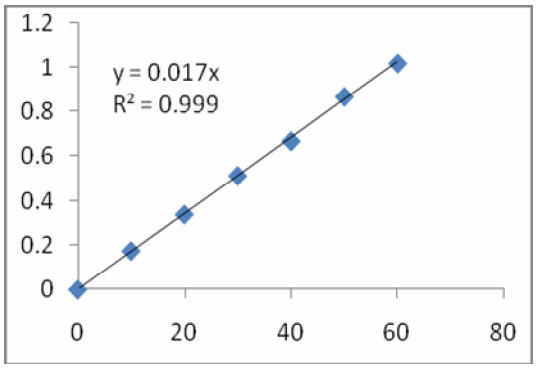


Table.1 Calibration Curve Table of LAM

Conc. In mcg/ml	**Absorbance**
10	0.1757
20	0.3401
30	0.5197
40	0.6706
50	0.8698
60	1.0206

Fig.2 Calibration Curve of TEN

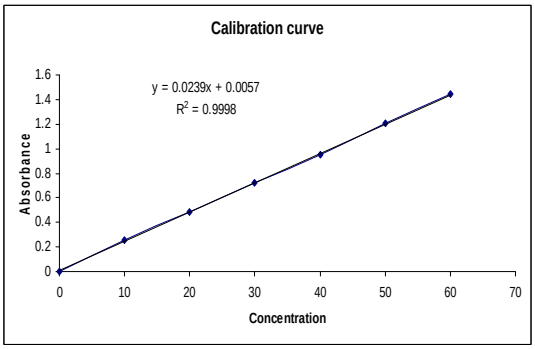
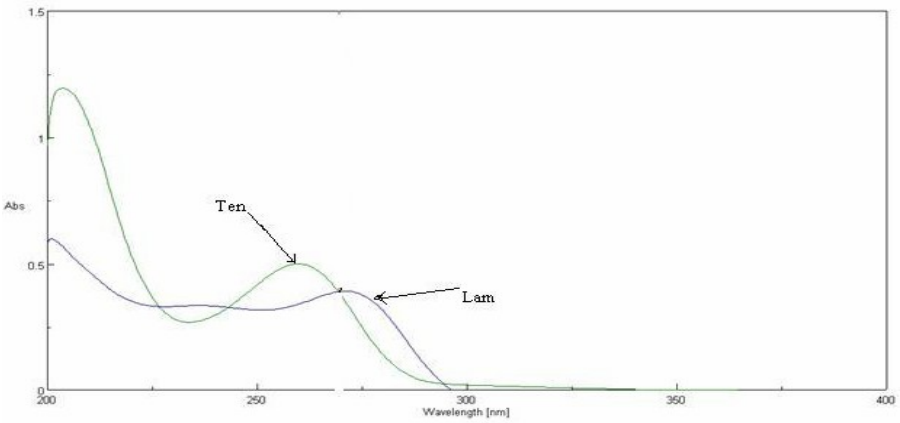


Table.2 Calibration Curve Table of TEN

Conc. in mcg/ml	Absorbance
10	0.2541
20	0.4858
30	0.7208
40	0.9507
50	1.2063
60	1.4404

Figure.4 Spectra showing Multicomponant mode



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