HPTLC Method for Simultaneous Estimation of Lamivudine and Zidovudine in Tablet Dosage Form

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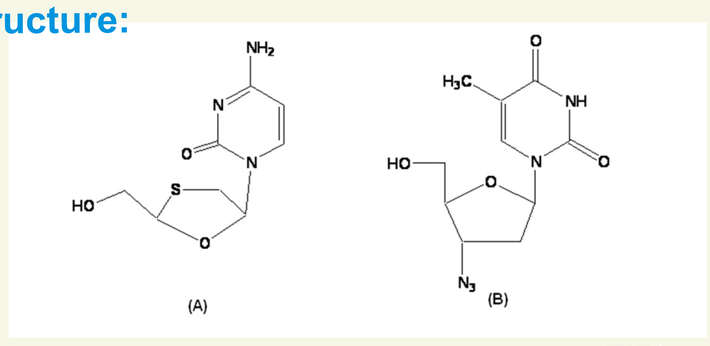
Drug Profile:

Structure:



(A) Lamivudine (LAM); (B) Zidovudine (ZID)

Category: Antiretroviral

Chemical Name:

(A) 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2(1H)-one
(B) 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)tetrahydro furan-2 yl]-5-methylpyrimidine-2,4(1H,3H)-dione

Empirical Formula: LAM - $C_8H_{11}N_3O_3S$; ZID - $C_{10}H_{13}N_5O_4$

Molecular Weight: LAM- 229.3; ZID- 267.2

Dissociation constant: LAM- 4.4; ZID- 9.7

Solubility: LAM- Water, Methanol; ZID- Methanol, Ethanol

Method Reported:

(A) Determination of Lamivudine by Capillary Electrophoresis[1], HPLC[2,3], LCMS[4,5] and HPTLC[6]
(B) Determination of Zidovudine by HPLC[7,8], LCMS[9]
(C) Simultaneous determination by UV- spectroscopy[10], MEKC[11], LCMS[12] and HPTLC[13]

Experimental:

Instruments: CAMAG LINOMAT-IV sample applicator with CAMAG TLC SCANNER III (Densitometer) and winCAT'S 4.0 version software

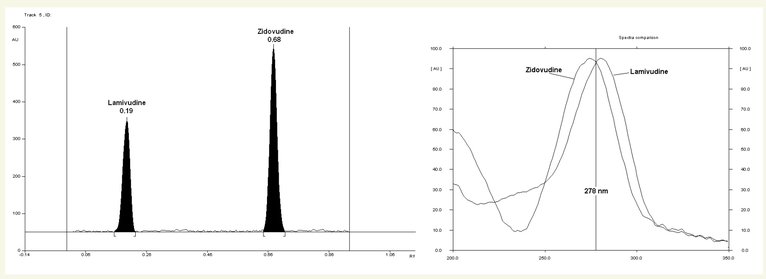
Reagents and Chemicals:

	Drug/Dosage form/Chemical	Manufacturer
Pure Drug Sample	Lamivudine (LAM)	Matrix Laboratories Ltd.
	Zidovudine (ZID)	Matrix Laboratories Ltd.
Tablet Formulation	*Cytocom*	Alkem Laboratories Ltd.
Chemicals	Chloroform, Methanol, Toluene	Qualigens
TLC Plate	Pre-coated silica gel G60, F_{254} HPTLC plates	E-Merck

Standard Solutions: 150 µg/mL of LAM, 300 µg/mL of ZID in methanol

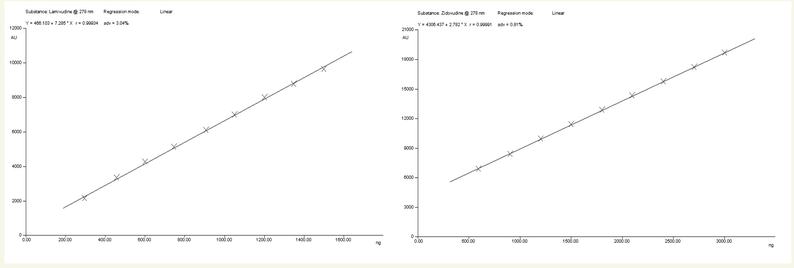
Chromatographic conditions:

Mobile phase : Chloroform: Methanol: Toluene [7.5:1.5:1 (v/v)]
Scanning wavelength: 278 nm
Stationary Phase : Aluminium precoated TLC plates Silica Gel G60, F254 TLC Plate, size 10 x 10 cm, 200 µm layer thickness
Mode of Application: Band
Band Width : 4 mm
Sample volume : 7 µL
Application rate : 5 sec/µL
Separation technique : Ascending
Development Chamber : Twin trough glass chamber, 10 x 10 cm.
Saturation Time : 15 min with mobile phase and spotted plate
Migration Distance : 80 mm.
Detection : UV Densitometric scanning
Scanning Mode : Absorbance/ Reflectance
Scanning speed : 20 mm/sec
Slit Dimension : 3 × 0.45 mm
Temperature : 25 ± 5ºC



Preparation of calibration curve:

Aliquot portions of working standard solution (1-12 µL) were applied on the TLC plate and densitograms were developed under optimized chromatographic conditions and the calibration curves were obtained. Linearity curves are shown in Figure 2. The curves were found to be linear between concentration range 300-1500 ng/spot for LAM and 600-3000 ng/spot ZID both by height and area. Results are summarized in Table 6.



Application of Proposed Method for Estimation in Marketed Formulation:

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 50.0 mg of LAM was transferred into a 50 mL volumetric flask containing little methanol. The powder dissolved in 30 mL methanol and the solution was sonicated for 30 min. The solution was cooled to room temperature and diluted up to the mark with methanol and filtered. A 3.75 mL of clear filtrate was transferred to a 25 mL volumetric flask and then volume was made up to the mark with methanol and used as working sample solution. Two bands of working standard and six bands of sample solution of equal volume (7µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions.

$$\%\ \text{Labelled claim} = \frac{Ew \times D \times Avg.Wt.}{Va \times Ws \times Lc} \times 100$$

Ew = Drug estimated in applied volume
D = Dilution factor
Va = Volume of sample applied
Ws= Weight of sample
Lc = Labelled claim of drug (mg/ml)

Table 1. Results of HPTLC Assay Studies

Component	Label claim (mg)	% of labeled claim* ± SD	% RSD
LAM	300	99.85 ± 1.2663	1.2681
ZID	150	99.84 ± 1.0948	1.0948

*Each value is a mean of five determinations

Validation of proposed method:

Precision:

Precision of estimation of LAM and ZID by proposed methods was ascertained by replicate analysis of homogenous samples of tablet powder.

Table 2. System, method and intermediate precision data

Formulation			By area	System Precision*	Method Precision*	Intermediate Precision*		
						Interday	Intraday	Different Analysts
CYTOCOM	LAM	Mean	99.88	99.31	99.70	99.77	99.48
		SD	1.0753	0.8937	1.1874	0.6834	1.1746
		% RSD	1.0766	0.9000	1.1909	0.6849	1.1808
	ZID	Mean	99.88	99.24	99.23	99.54	99.27
		SD	1.1767	0.9392	1.2875	0.6352	0.9868
		% RSD	1.1781	0.9464	1.2975	0.6381	0.9941

*Each value is a mean of six determinations

Accuracy:

Accuracy of Proposed method was ascertained on the basis of recovery studies were carried out by standard addition method.

Table 3. Results from recovery analysis

CYTOCOM Tablet (Avg. wt. 172.23 mg)						
Sr. No.	% Spiking Level	Wt. of sample + std. LAM# + std. ZID# (mg)	Amount of std. drug recovered by area (mg)*		% Recovery*	
			LAM	ZID	LAM	ZID
1	80	198.24 + 5.0 + 10.0	4.89	9.89	97.80	98.90
2	100	197.43 + 15.0 + 30.5	15.03	30.21	100.20	100.70
3	120	196.04 + 25.0 + 50.0	24.87	48.97	99.48	97.94
				Mean	99.16	99.18
				SD	1.2316	1.4011
				% RSD	1.2420	1.4127

*Each value is a mean of five determinations,
#Added in the form of standard stock solution

Specificity:

The specificity of the method was ascertained by how accurately and specifically the analyte of interest are estimated in the presence of other components (e.g. impurities, degradation products, etc.) by exposing the sample to different stress conditions such as acidic (0.1 N HCl), alkaline (0.1N NaOH), oxidizing (3% H_2O_2), heat (60ºC) and UV radiations for 24 h and then analyzing them by proposed method.

Table 4. Results of Specificity

Sr. No.	Sample	% Labeled claim by area	
		LAM	ZID
1.	Normal	100.67	100.91
2.	Acid	83.61	93.77
3.	Alkali	91.25	85.19
4.	Oxide	79.90	83.73
5.	Heat	99.78	99.35
6.	Sunlight	78.82	94.17

Ruggedness:

It is a degree of reproducibility of test results obtained by the analysis of the same samples under variety of conditions such as different laboratories, different analyst and different instruments and different days. Results are shown in table 2.

Robustness:

It is the ability of the analytical method to remain unaffected by small but deliberate variation in method parameter and provide its reliability during normal usage.

Table 5. Results of Robustness

Method Parameter		LAM			ZID		
		Mean*	SD	%RSD	Mean*	SD	%RSD
Wavelength	276 nm	99.61	1.3341	1.3394	98.66	0.6156	0.6240
	280 nm	99.07	0.7986	0.8061	99.37	0.6705	0.6747
Temperature	22°C	98.42	0.5230	0.5314	99.50	0.8138	0.8179
	28°C	99.40	0.8856	0.8910	98.87	0.6573	0.6647
Saturation period	8 min	98.71	0.4750	0.4812	100.05	0.9753	0.9748
	12 min	99.12	0.7642	0.7710	99.95	1.1764	1.1770

LOD & LOQ:

Table 6. Analytical Performance Data

Parameters	LAM		ZID	
	By height	By area	By height	By area
Linear dynamic range (ng/band)	300–1500	300–1500	600–3000	600–3000
Slope	0.233	7.285	0.104	2.782
Y-intercept	46.108	466.103	229.632	4306.437
Correlation coefficient (r)	0.998	0.999	0.998	0.999
LOD (µg/mL)	84.98	49.83	491.83	385.51
LOQ (µg/mL)	257.51	150.99	1490.39	1168.22

Results and Conclusion:

Results of marketed formulation of LAM and ZID were found to be 99.85±1.2663 and 99.84±1.0948 respectively.
The average recovery values are obtained were 99.16±1.2316 and 99.18±1.4011.
The proposed method is simple fast cost effective and therefore can be applied for routine quality control of pharmaceutical preparations.

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Acknowledgement:

The authors extend their sincere thanks to Matrix Laboratories Ltd., Hyderabad, India and for providing gift sample of pure lamivudine and zidovudine. We also extend our thanks to Head of Department, Department of Pharmaceutical Sciences; RTM Nagpur University for providing the necessary facilities.