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A Validated HPTLC Method for Simultaneous Estimation of Lamivudine and Zidovudine in Tablets

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A validated high performance thin layer chromatographic method for simultaneous estimation of lamivudine and zidovudine in tablets is described. Aluminium plates precoated with silica gel 60 F_{254} were used as stationary phase and a mixture of toluene : methanol : *n*-hexane (7 : 1.5 : 1.0) (v/v) as mobile phase. Quantitation was carried out by the use of densitometer in absorbance mode at 275 nm. Linearity of detector response for lamivudine and zidovudine was found in the range of 0.8–2.0 and 1.5–4.0 μg, respectively. Amounst of lamivudine and zidovudine estimated in the average weight of the tablet were found to be 149.09/148.12 and 301.41/300.74 mg (as per peak height/area), respectively. The per cent recovery for lamivudine and zidovudine was found to be 100.31/99.19 and 100.01/99.43% (as per peak height/area), respectively. The proposed method is accurate, precise, specific and reproducible and can be adopted for routine analysis of lamivudine and zidovudine in tablet formulation.

Key Words: Lamivudine, Zidovudine, Thin layer chromatography, Validation.

INTRODUCTION

Lamivudine[1] is chemically (2R-*cis*-4-amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2-(1H)-pyrimidinone while zidovudine[2] is 3-azido-3′-deoxythymidine. Both the drugs are available in combination as tablet, used for treating HIV infection. Literature survey reveals that lamivudine is estimated by spectrophotometry[3, 4] and HPLC[5, 6]. Zidovudine is reported to be estimated by spectrophotometry[7], HPLC[8, 9] and HPTLC[10]. These drugs in combination have been analyzed by HPLC[11, 12] method. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by an accurate, precise, specific, economical and less time consuming high performance thin layer chromatography (HPTLC) method.

EXPERIMENTAL

Camag-HPTLC system comprises Camag Linomat IV automatic sample applicator, Camag TLC scanner 3 with CAT'S 4 software for interpretation of data, Camag twin trough glass chamber. Toluene, methanol, *n*-hexane were of HPLC grade purity.

Chromatographic conditions

Stationary phase: Aluminium plates precoated with silica gel 60 F_{254} (10 cm

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× 10cm). Mobile phase: toluene : methanol : n-hexane (7 : 1.5 : 1.0 v/v). Saturation time: 10 min. Application mode: band (4 mm). Injection volume: 5 µL. Separation technique: ascending development. Migration distance: 70 mm. Temperature: 25 ± 5°C. Relative humidity: 50–60%. Scanning mode: absorbance/reflectance. Lamp: deuterium. Wavelength: 275 nm, selected from overlain sectra of both the drugs (Fig. 1). Slit dimension: 3 × 0.45 mm.

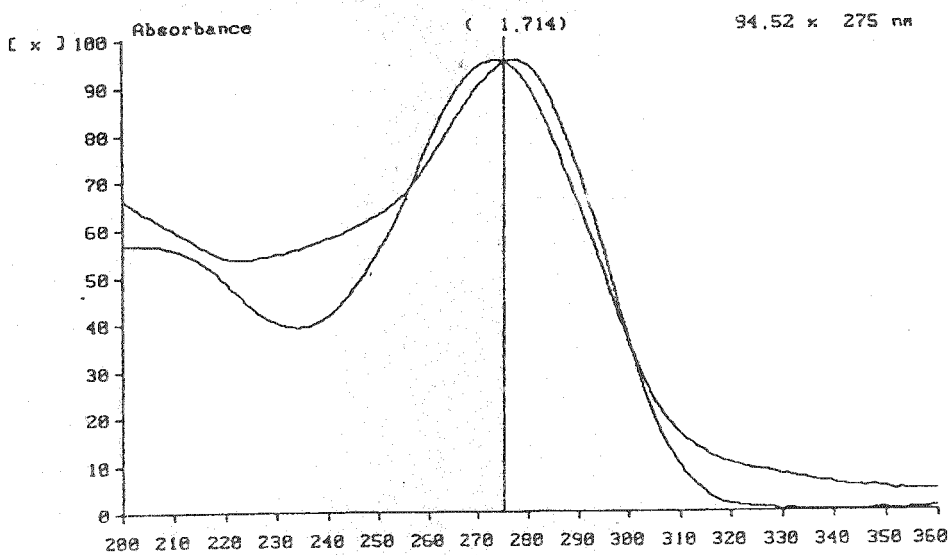

Fig. 1

Preparation of standard solution

Standard stock solution: A mixed standard solution containing 0.5 mg/mL of lamivudine and 1 mg/mL of zidovudine was prepared in methanol.

Standard solution: The standard stock solution was diluted with methanol to get a final concentration of 250/500 ng/mL of lamivudine/zidovudine.

RESULTS AND DISCUSSION

Linearity and calibration

Aliquots of standard solution were applied on a TLC plate with the help of Camag Linomat IV auto sampler. The chamber was allowed to saturate with mobile phase. The mobile phase was then allowed to run on TLC plate. After development, the plate was removed from the chamber and allowed to air dry. The spots of lamivudine and zidovudine were evaluated at 275 nm. The plot of peak height/area *vs.* the respective concentration of lamivudine and zidovudine were found to be linear in the range of 0.8–2.0 and 1.5–4.0 µg, respectively.

Assay: Twenty tablets were weighed and finely powdered. Accurately weighed quantity of tablet powder equivalent to about 25 mg of lamivudine and 50 mg of zidovudine was transferred to a 50 mL volumetric flask, added 25 mL of methanol and shaken for 5 min; the volume was then made up to the mark with methanol. The solution was filtered through Whatmann filter paper and the filtrate was appropriately diluted with methanol to get a final concentration of about 250/500 µg/mL of lamivudine/zidovudine. The resulting solution was used for

analysis. Chromatograms were obtained by maintaining the above said chromatographic conditions and evaluation was performed using peak height and peak area. The results of estimation are shown in Table-1.

TABLE- 1
RESULTS OFESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN TABLET FORM

Spl. code	Label claim (mg/tab)	Statistics	Amount of drug estimated* (mg/tablet)				Labelled claim* (%)			
			Lamivudine		Zidovudine		Lamivudine		Zidovudine	
			Peak height	Peak area	Peak height	Peak area	Peak height	Peak area	Peak height	Peak area
T-1	Lam-150 ZID-300	Mean	148.33	148.19	300.84	300.60	99.02	98.99	100.28	100.20
		S.D.					±0.84	±0.57	±0.88	±0.87
		C.V.					0.85	0.58	0.88	0.87
T-2	Lam-150 ZID-300	Mean	149.65	148.04	301.97	300.87	99.77	98.69	100.66	100.29
		S.D.					±0.80	±0.77	±0.85	±0.65
		C.V.					0.80	0.78	0.84	0.65

*Mean of five observations.

Validation

Accuracy of the proposed method was ascertained on the basis of recovery studies carried out at four different levels by standard addition method. The per cent recovery was calculated by using the following formula: T-A/S × 100 where T is total amount of drug estimated, A is amount of drug contributed by tablet powder (as per the amount estimated by proposed method) and S is the amount of pure drug added. The results of recovery studies are shown in Table-2.

Precision of the analytical method is expressed as S.D. or C.V. of series of measurements by replicate estimation of the drugs by proposed method (Table-1).

TABLE-2
RESULTS OF RECOVERY STUDIES

S. No.	A mount of drug added (mg)		Amount of drug estimated* (mg/tablet)				Labelled claim* (%)			
			Lamivudine		Zidovudine		Lamivudine		Zidovudine	
	LAM	ZID	Peak height	Peak area	Peak height	Peak area	Peak height	Peak area	Peak height	Peak area
1.	25.3	51.0	24.45	24.93	50.59	51.67	100.59	98.54	99.20	101.31
2.	19.9	42.0	20.08	19.64	41.78	42.02	100.91	98.69	99.48	100.05
3.	15.6	29.7	15.80	15.32	29.52	29.20	101.28	98.21	99.39	98.32
4.	9.8	20.2	9.65	9.93	20.60	19.80	98.47	101.33	101.98	98.02
			Mean per cent recovery				100.31	99.19	100.01	99.43
						S.D.	±1.09	±1.25	±1.14	±1.34
						C.V.	1.09	F1.26	1.14	1.35

The specificity studies were carried out by deliberately degrading the marketed sample. The stress conditions applied were acidic condition (0.1 M HCl), alkaline condition (0.1 M NaOH), oxidizing condition (3% H_2O_2) for 24 h at 50°C. Also, heat (60°C) and UV exposure for 24 h was studied.

The proposed method gives good resolution of lamivudine and zidovudine with the R_f values of 0.13 and 0.28, respectively. The per cent of lamivudine and zidovudine estimated in the average weight of tablet was found to be around 99 and 100% of labelled claim, respectively. The lower values of standard deviation and coefficient of variance for assay indicate high precision of the method. The mean per cent recoveries of lamivudine and zidovudine were in the range of 99.19 and 100.31% indicating that the method is specific, accurate and free from interference of excipients present in the formulation. The results obtained for specificity studies of lamivudine and zidovudine (as per peak height/area) under different stress conditions are as follows: acidic (91.93/93.13 and 100.61/98.97%), alkaline (94.34/92.99 and 97.17/97.09%), oxidizing (quantitation of lamivudine is not possible due to change in R_f value, zidovudine: 95.38/97.01%), heat (99.86/97.94 and 102.67/101.98%), UV exposure (93.39/97.36 and 99.59/102.84%).

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REFERENCES

1. The Merck Index, 12th Edn., Merck Research Lab., Division of Merck and Co., Inc., Whitehouse Station, NJ, p. 914 (1996).
2. The United States Pharmacopoeia, 25th Edn., United States Pharmacopoeial Convention Inc., Rockville (1821).
3. M.V. Baig, G.S. Kapse and S.A. Raju, *Asian J. Chem.*, **13**, 185 (2001).
4. V. Rajasekaran, V. Murugesan, K.A. Rajagopal, A.G.I. Blessing, T.N. Chakravarthy and D.S.I. Pushpraj, *Orient. J. Chem.*, **16**, 563 (2000).
5. X.J. Zhou and J.P. Sommadossi, *J. Chromatogr. B*, **691**, 417 (1997).
6. A.J. Harker, G.L. Evans, A.E. Hawley and D.M. Morris, *J. Chromatogr. B*, **657**, 227 (1994).
7. V.V. Nesterov, N.S. Evtushenko, A.V. Titova and V.Yu. Reshetnyak, *Farmatsiya*, **40**, 30 (1991).
8. M.A. Radwan, *Anal. Lett.*, **27**, 1159(1994).
9. S.S. Zarapkar, B. Salunkhe and U.B. Salunkhe, *Indian Drugs*, **28**, 434 (1991).
10. H. Tomankova and J. Sabartova, *Chmmtitttgraphia*, 29,44 (1990).
11. K.B. Kenney, S.A. Wring, R.M. Carr, G.N. Wells and J.A. Dunn, *J. Pharm. Biomed. Anal.*, **22**, 967 (2000).
12. P.J. Wagh and N. Pai, *Indian Drugs*, **39**, 657 (2002).

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