Simultaneous High Performance Thin Layer Chromatographic Estimation of Lamivudine and Stavudine in Tablet Dosage Forms

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The present work describes a validated high performance thin layer chromatographic method for simultaneous estimation of lamivudine and stavudine in tablet formulation. Silica gel 60 F_{254} plate were used as stationary phase and toluene:n-hexane:tetrahydrofuran (7:1.5:1.5 v/v) as mobile phase. The wavelength selected for analysis was 253 nm. Amount of lamivudine and stavudine estimated as per peak height/area were found to be 149.9/147.5 mg and 30.2/30.6 mg, and the percentage recoveries for both the drugs were 99.4/98.8 % and 99.3/100.0 %, respectively.

Lamivudine[1] [(-)-2'-deoxy-3'-thiacytidine] and stavudine[1] [1-(2,3-Dideoxy-β-D-glycero-pent-2-eno-furanosyl) thymine] are nucleoside reverse transcriptase inhibitors used for treating HIV infections. Fixed dose combination tablet containing lamivudine (150 mg) and stavudine (30 mg) is available for clinical use. Both drugs are official in Martindale, the Extra pharmacopoeia. Literature survey revealed spectrophotometric[2,3], and HPLC[4,5] methods for estimation of lamivudine and stavudine individually in pharmaceutical formulations and biological samples. HPLC[6] method has been reported for simultaneous estimation of these drugs along with nevirapine in tablets. In the present work a successful attempt has been made to estimate both these drugs by accurate, sensitive, economical and less time-consuming HPTLC method.

A Camag-HPTLC system comprising of Linomat IV automatic sample applicator and Camag-TLC scanner 3 with CAT'S version 4.0 software were used for sample application and quantitative evaluation respectively. Samples were applied as bands 4 mm width and at 4 mm intervals under a stream of nitrogen on aluminum plates coated with silica gel 60 F_{254} (10×10 cm, Merck) and chromatographer using toluene:n-hexane:tetrahydrofuran (7:1.5:1.5 v/v) as mobile phase. Ascending development was performed in a saturated twin-trough TLC chamber. Chromatogram was evaluated by scanning in absorbance/reflectance mode at 253 nm using slit dimensions 3×0.45 mm and quantitation was done using peak height and peak area.

Standard stock solution containing 1 mg/ml of lamivudine and 0.2 mg/ml of stavudine was prepared by dissolving 50 mg lamivudine and 10 mg stavudine in 50 ml methanol. The linear detector response for lamivudine and stavudine was observed between 2.5-7.4 μg and 0.5-1.4 μg, respectively.

For estimation of lamivudine and stavudine in tablets, an accurately weighed quantity of tablet powder equivalent to 50 mg lamivudine and 10 mg stavudine were transferred to a 50 ml volumetric flask, shaken with 25–30 ml methanol for 10 min and the volume was then adjusted to the mark with methanol. The solution was then filtered through Whatman filter paper No. 41 and 5 μl of the filtrate was applied on HPTLC plate and chromatogram was run. The amounts of both the drugs were estimated by comparing the peak height and peak area of standard and sample spots. The results are shown in Table 1. Representative densitogram is shown in fig. 1.

To study the accuracy and precision of the method recovery studies were carried out using standard addition method at four different levels. The percent recovery was calculated by using the formula, % recovery=(T–A)/S×100, where, T is total amount of the drug estimated, A is the amount of drug contributed by tablet powder, and S is the amount of pure drug added. The results of recovery studies are shown in Table 1. The percent recovery for both the drugs

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TABLE 1 : RESULTS OF ESTIMATION IN TABLET AND RECOVERY STUDIES.

Sample code	Label Claim (mg/tab)	% label claim*				% recovery			
		LAM		STA		LAM		STA	
		Height	Area	Height	Area	Height	Area	Height	Area
Standard laboratory mixture	LAM 150 mg STA	99.7±.8	99.6±.7	101±1.0	101±.8	98.8	98.9	99.0	102
						98.6	100	98.2	98.0
Tablet formulations	30mg	100±.5	98.4±.6	101±1.1	102±.4	99.4	98.1	98.4	102
						101	98.5	101	98.1
Mean±SD						99.5±.8	98.8±.5	99.1±1	100±2

The abbreviation LAM stands for lamivudine and STA stands for stavudine. The '*' mark on % of label claim indicates mean of five observations and the '±' sign indicates standard deviation of five observation.

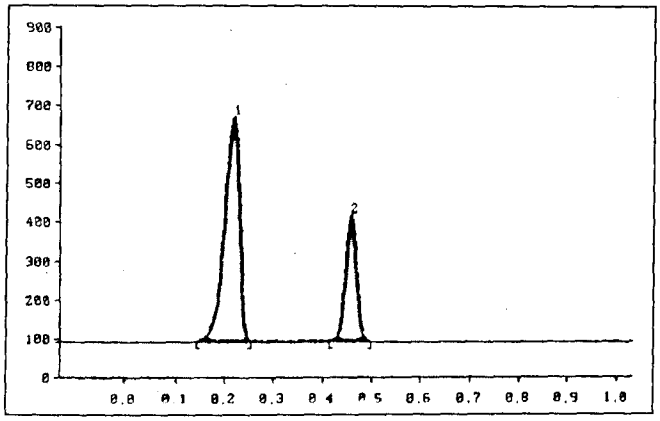

Fig. 1: chromatogram of marketed formulation

1 - Lamivudine and 2 - Stavudine

was found to be around 99 % indicating that the method is free from interference from the excipients.

The system repeatability was studied by applying five replicate applications of standard stock solution to the HPTLC plate. The standard deviation for peak height/area was ±0.04/0.10 for lamivudine and ±0.03/0.02 for stavudine, respectively. The robustness of the method was evaluated by studying analyst to analyst, intra and inter day variations. The SD for analyst to analyst, intra and inter day variation was below 2 %. The specificity studies were carried out by deliberately degrading the tablet sample. The stress conditions applied were heat (60°), acidic condition (0.1 M HCl), alkaline condition (0.1 M NaOH), oxidizing condition (3% H_2O_2), and UV-light exposure for 24 h . The results obtained for lamivudine and stavudine as per peak height/area in different stress conditions were, heat (98.2/96.4 % and 97.3/101.6 %), acidic (90.5/106.6 and 85.5/84.8 %), alkaline (95.7/92.5 % and 73.5/82.0 %), oxidizing (11.6/4.4 % and 86.1/93.6 %), and UV-exposure (99.4/96.6 % and 98.1/96.9 %). From the above results it can be concluded that the proposed method is accurate, precise, specific, and reproducible and can be used for routine analysis of lamivudine and stavudine in tablet formulation.

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