
Concurrent Assay of Lamivudine and Zidovudine from Combination Tablets

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A simple, fast and precise reversed phase high performance liquid chromatographic method developed for the simultaneous determination of lamivudine and zidovudine in tablets. A μ Bondapak C₁₈, 10 μ m column (300x3.9 mm i.d.) was used in isocratic mode, with mobile phase composed of 0.02 M tri-sodium citrate and methanol in the ratio of 70:30. The flow rate was 1.0 ml/min and effluent was detected at 266 nm. The peaks were examined for chromatographic figures of merit. Peak heights were measured as detector responses. The linear dynamic ranges for lamivudine and zidovudine were found to be 12-18 μ g/ml and 24-36 μ g/ml, respectively. Analytical performance parameters like precision, accuracy, etc. were determined to assure the viability of the method. After validation, the method was applied to the assay of drugs in a combination tablet. Application of the method revealed that the content of lamivudine was 149.6 \pm 0.47 mg against labeled amount of 150 mg and zidovudine was 299.1 \pm 0.65 mg against labeled amount of 300 mg (n=7).

Lamivudine is (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone, while zidovudine is 3'-azido-3'-dioxymethylene-2,3-dihydro-2H-pyrimidin-4(1H)-one. Both are phosphorylated in the body to their active triphosphate form. Lamivudine inhibits the hepatitis B virus polymerase and reverse transcriptase enzyme of HIV. Zidovudine inhibits the key enzyme reverse transcriptase¹. Both are used in combination in treatment of HIV infections at recommended dose of 150 and 300 mg, respectively. The literature survey reveals only one HPLC method for the chiral separation of lamivudine², while individual estimation of zidovudine is reported by HPLC in two similar publications^{3,4}. However, there is no method reported so far for simultaneous determination of these two drugs from the formulations by RP-HPLC. The present paper reports the isocratic separation and estimation of the two drugs in bulk and a tablet formulation.

Methanol of HPLC grade was obtained from E. Merck Ltd, Mumbai. Tri-sodium citrate (AR) was obtained from S. D. Fine Chem Ltd, Mumbai. Working standards of lamivudine and zidovudine were supplied by Kopran Pharmaceutical

Limited as standard certified samples. Locally available commercial dosage form tablets (Duovir, Cipla Ltd) were used for analysis.

Separate stock solutions of the drugs were prepared by dissolving 100 mg each in 100 ml of methanol. Mixtures of the drug solutions were prepared by pipetting out appropriate quantities of the individual stock solutions and then diluting to 10 ml with mobile phase. A Jasco-1580 series LG-1580-02 Ternary Gradient HPLC chromatograph equipped with PU-1580 Intelligent HPLC pump, AS-1555-10 Intelligent auto sampler and UV-1575 Intelligent UV/Vis detector was used. For obtaining optimum parameters for the baseline resolution and ideal chromatographic figures of merit for the two drugs, 20 μ l of the individual drug solutions at concentrations of 15 μ g/ml of lamivudine and 30 μ g/ml of zidovudine were injected into a μ Bondapak C₁₈ (300x3.9 mm) 10 μ m column at 1.0 ml/min flow rate of mobile phase. Mobile phase was prepared by mixing 70 parts of 0.02 M tri-sodium citrate and 30 parts of methanol. The analytes were detected at 266 nm. Fig. 1 shows the typical chromatogram of a mixture of lamivudine and zidovudine under optimized conditions.

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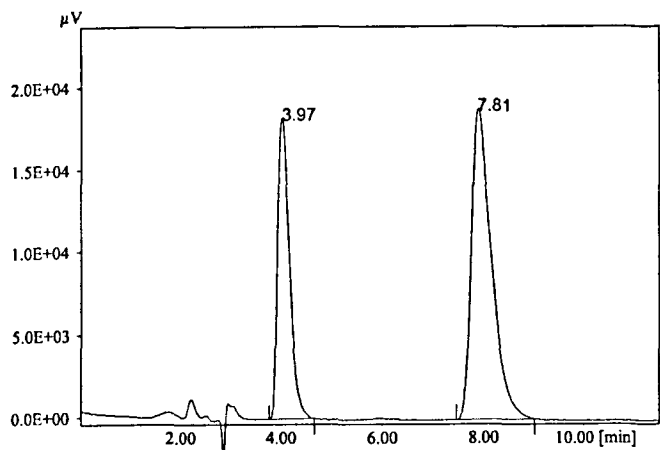


Fig. 1: Typical chromatogram of a mixture of lamivudine and zidovudine.

Typical chromatogram of a mixture of lamivudine (15 $\mu\text{g/ml}$, $t_R = 3.97$ min) and zidovudine (30 $\mu\text{g/ml}$, $t_R = 7.80$ min) under optimized conditions.

Chromatographic parameters like retention time (t_R , min), capacity factor (k'), selectivity (α), resolution (R), number of theoretical plates (N), peak tailing factor (T) and HETP (height equivalent to theoretical plates, h cm) for these two analytes were calculated and are given in Table 1.

For linearity studies, different concentrations of lamivudine (12 to 18 $\mu\text{g/ml}$) and zidovudine (24 to 36 $\mu\text{g/ml}$) were assayed ($n=5$). The detector response (peak height) was found to be rectilinear over the concentration ranges studied. The data was analyzed by the linear regression least squares fit method and the statistical data are presented in Table 2.

A study of accuracy and precision was performed by assaying three composite solutions of low, medium and high concentrations of the mixtures of these drugs. The peak responses in terms of peak height were then related to the slopes and intercepts mentioned in Table 2 to obtain analytical recoveries. Table 3 lists the recoveries of the drugs from a series of spiked concentrations and shows that the relative standard deviations (RSD) do not exceed 2 % for these drugs.

Well known brand, Duovir a combined dosage form of lamivudine (150 mg) and zidovudine (300 mg) was obtained locally. Twenty tablets were powdered and homogenized. Portions to obtain labeled dose were weighed out and dissolved in 100 ml of methanol. The solutions were filtered and diluted to conform to the linear dynamic regions of the drugs given in Table 2. Twenty microlitres of this solution

TABLE 1: CHROMATOGRAPHIC FIGURES OF MERIT RECORDED IN THE ANALYSIS.

	Lamivudine	Zidovudine
Retention time (t_R)	3.97	7.81
Relative retention time (t_R)	1.00	1.97
Capacity factor (k')	0.20	4.83
Selectivity factor (α)	-	2.42
Resolution (R)	-	5.03
No. of plates (N)	685	1225
Tailing factor (T)	1.01	1.17
H. E. T. P. (Height equivalent to theoretical plates, h cm)	0.04	0.024

Figures denote ideal chromatographic separation, but only the efficiency is lower.

TABLE 2: LINEAR REGRESSION (LEAST SQUARES FIT) CALIBRATION DATA.

Drug	Lamivudine	Zidovudine
Linear dynamic range ($\mu\text{g/ml}$)	12-18 $\mu\text{g/ml}$	24-36 $\mu\text{g/ml}$
Slope (m)	1174.0	577.6
Intercept (b)	-211.4	95.3
S.D. of slope (S_m)	5.29	2.53
S.D. of intercept (S_b)	80.1	76.7
Correlation coefficient (r)	0.9999	0.9999

$y=mx+b$, where y = peak response, m = slope, x = concentration ($\mu\text{g/ml}$), b = intercept.

was injected into the chromatograph under the conditions specified. The analyte peaks were identified by comparison with observed retention times with those of respective standards. From a series of seven experiments, the mean contents of the tablets were found to be 149.6 ± 0.47 mg and 299.1 ± 0.65 mg of lamivudine and zidovudine, respectively, as against 150 mg and 300 mg labeled amount respectively. Commonly used excipients like starch, micro-crystalline cellulose, talc, lactose, HPMC, stearic acid, boric acid, gelatin, magnesium carbonate, sodium lauryl sulphate do not interfere in the method, as was seen from the equality of responses from dosage form and the synthetic mixtures of the

TABLE 3: ACCURACY AND PRECISION OF THE METHOD – MEAN VALUES FOR SIMULTANEOUS ASSAY OF TWO DRUGS.

	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	Error (%)	Recovery (%)
Lamivudine	12	0	12	11.9	0.83	99.16
	12	3	15	14.9	0.66	100.6
	12	6	18	17.9	0.55	99.44
Zidovudine	24	0	24	23.9	0.41	99.58
	24	6	30	29.9	0.33	99.66
	24	12	36	36.2	0.55	100.5

% Error=[Difference in conc. added and conc. found/conc. added]x100

drugs incorporating these excipients.

A simple, sensitive method has been developed for the isocratic separation and simultaneous estimation of lamivudine and zidovudine in bulk and tablet dosage form. Hence it can be conveniently adopted for the routine quality control analysis.

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Simultaneous HPTLC Determination of Rifampicin and Isoniazid in Rat Plasma

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A method enabling the precise and quick simultaneous assessment of rifampicin and isoniazid in rat plasma by HPTLC has been presented. Extracted samples of rifampicin and isoniazid in organic solvents were separated on a plate coated with silica gel 60 F₂₅₄, chromatograms were developed using a mixture of chloroform:methanol and quantification was carried out by the use of densitometer absorbance mode at 475 nm and 280 nm respectively. Under the operating conditions, the lower limit of detection was found to be 0.3 µg/spot for rifampicin and 0.1 µg/spot for isoniazid. The method has a linearity range of 10 to 80 µg/ml for both the drugs and recovery was found to be 97.5% for both rifampicin and isoniazid. The pharmacokinetic parameters obtained after intravenous bolus injection at a dose of 10 mg/kg has also been presented.

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