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Simultaneous Estimation of Lamivudine, Zidovudine and Nevirapine by RP-HPLC in Pure and Pharmaceutical Dosage Form

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A simple, accurate, precise and reproducible high performance liquid chromatographic method has been developed for the simultaneous estimation of lamivudine, zidovudine and nevirapine in pharmaceutical dosage forms. A Gemini ODS C_{18} column (4.6 mm × 25 cm i.d., 5 µm particle size) in isocratic mode, with mobile phase acetonitrile, orthophosphoric acid buffer (pH 3.5) and methanol (15:60:25) (v/v/v) the flow rate was 1 mL/min and effluent was monitored at 265 nm. The approximate retention time for lamivudine, zidovudine and nevirapine were 3.36, 5.30 and 9.28 min, respectively. The linearity for lamivudine, zidovudine and nevirapine was in the range of 36-84, 72-168 and 48-112 µg/mL, respectively. Quantity found for lamivudine, zidovudine and nevirapine were 150.6, 298.11 and 199.12 mg, respectively. The percentage estimation of labeled claims of lamivudine, zidovudine and nevirapine from marketed tablet was found to be 100.40, 99.37 and 99.55, respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. The addition of known quantity of standard drugs in the pre-analyzed test solution percentage recovery was calculated in each case. The percentage recoveries obtained for lamivudine, zidovudine and nevirapine were found within the range of 98.62-99.88 %. The proposed method is found to be accurate, precise, simple and rapid which can be used routinely for simultaneous estimation of proposed combination in tablet formulation.

Key Words: HPLC, Lamivudine, Zidovudine, Nevirapine, Tablets.

INTRODUCTION

Zidovudine[1,2] is an orally administered thymidine derivative with effects for the management of HIV infection. It is from the class of nucleoside reverse transcriptase inhibitor. Chemically, 3'-azido-2',3'-di deoxythymidine. Its empirical formula is $C_{10}H_{13}N_5O_4$ and molecular weight 267.242. The mode of action is by terminating the growth of the DNA chain and inhibiting the reverse transcriptase of HIV.

Lamivudine[1,2] is an orally administered. It also belongs to the class of nucleoside reverse transcriptase inhibitor. Chemically, lamivudine is L-2',3'-dideoxy-3'-thiacytidine. Its empirical formula is $C_8H_{11}N_3O_3S$ and molecular weight is 229.26. It acts by competes with deoxycytidine triphosphate for binding to reverse transcriptase and incorporation into DNA results in chain termination.

Nevirapine[1,2] is an orally administered antiretroviral drugs. It belongs to the class of non-nucleoside reverse transcriptase inhibitor (NNRTI). Chemically, nevirapine is 1-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-b: 2', 3'-e][1,4]diazepin-6-one. Its empirical formula is $C_{15}H_{14}N_4O$ and molecular weight is 266.298. It acts by binding to reverse transcriptase adjacent to the catalytic site and terminate the DNA chain.

The literature survey[3-6] reveals the analytical methods like UV, HPLC and HPTLC for determination of these drugs individually and other combinations in pharmaceuticals and biological preparations. In the present investigation an attempt was made to develop a simple, accurate, sensitive and economical HPLC for the simultaneous estimation of lamivudine, zidovudine and nevirapine in tablet dosage forms.

EXPERIMENTAL

High performance liquid chromatograph Shimadzu LC 2010CHT series equipped with quaternary constant flow pump, auto injector with injection volume of 20 µL. Photo diode Array detector and LC 10 software, Gemini ODS C18 column (4.6 mm × 25 cm i.d., 5 µm particle size) forms the stationary phase, a calibrated electronic single pan balance (Mettler AE 160) and certified reference standards of lamivudine, zidovudine and nevirapine were used. The drug samples were procured from the market. Tablets claim for lamivudine, zidovudine and nevirapine were 150, 300 and 200 mg/tablet, respectively. All chemicals and reagents used were of AR/HPLC grade. HPLC grade water was prepared by Millipore water purification system (Milli-Q) in the lab.

Preparation of standard solution: An accurately weighed quantity of lamivudine (150 mg), zidovudine (300 mg) and nevirapine (200 mg) were transferred to 10 mL volumetric flask, which was then dissolved and made up to volume with mobile phase. From the above stock solution 1.2, 1.6, 2.0, 2.4, 2.8 mL of lamivudine, zidovudine and nevirapine were diluted to 50 mL with mobile phase to give final concentration of 36, 48, 60, 72 and 84 µg/mL of lamivudine, 72, 90, 120, 144 and 168 µg/mL of zidovudine and 48, 64, 80, 96 and 112 µg/mL of nevirapine. The solutions were injected and chromatograms were recorded.

Optimized chromatographic conditions: HPLC analysis was performed by isocratic elution with flow rate of 1.0 mL/min. The mobile phase

containing acetonitrile, orthophosphoric acid buffer (pH 3.5) and methanol (15:60:25) (v/v/v) to obtain well-resolved peaks. Injection volume of 20 µL each of standard and sample solutions were injected into the column containing stationary phase octyl decyl silane with particle size of 5 µm. The detection wavelength and chromatographic run time was selected at 265 nm and 15 min, respectively.

Calibration curve: Linearity was assessed by injecting 20 µL of seven different standard concentrations obtained by diluting standard stock solution with mobile phase under optimized chromatographic conditions, which provides 36, 48, 60, 72 and 84 µg/mL of lamivudine, 72, 90, 120, 144 and 168 µg/mL of zidovudine and 48, 64, 80, 96 and 112 µg/mL of nevirapine. The chromatograms were recorded and using peak area of individual drugs *vs*. respective concentrations linearity graph was plotted.

Sample preparation: 20 Tablets were weighed accurately and crushed to fine powder. An accurately weighed quantity of powder equivalent to 150 mg of lamivudine, 300 mg of zidovudine and 200 mg of nevirapine was then transferred to a 100 mL volumetric flask, sonicated for 15 min and made up to volume with mobile phase. Further the solution was filtered through Whatmann filter paper no. 4, which was used for determining the content of lamivudine, zidovudine and nevirapine simultaneously in conventional conditions.

Estimation method: With the optimized chromatographic conditions mentioned earlier, a steady base line was recorded. After the stabilization of baseline, successive aliquots of standard solutions, which have been prepared from stock solutions, containing varying concentrations were injected and chromatograms were recorded. The procedure was repeated using sample solutions having different concentrations which was prepared by diluting aliquot of sample stock solution to get final concentrations of 36, 48, 60, 72 and 84 µg/mL of Lamivudine, 72, 90, 120, 144 and 168 µg/mL of zidovudine and 48, 64, 80, 96 and 112 µg/mL of nevirapine.

$$\text{Amount of drug in tablet} = \frac{\text{Peak area of test compound} \times \text{conc. of std.} \times \text{avg. wt.} \times (100\text{-std. LOD}) \times \text{std. purity}}{\text{Peak area of STD} \times \text{conc. of sample}}$$

RESULTS AND DISCUSSION

The method was chosen after several trials with various proportions of acetonitrile, orthophosphoric acid buffer (pH 3.5) and methanol and at different pH values. A mobile phase consisting of acetonitrile, orthophosphoric acid buffer (pH 3.5) and methanol in the ratio of (15:60:25) (v/v/v) was selected to achieve maximum separation and sensitivity. The effects of flow rates in the range of 0.5-1.5 mL/min were examined where the

flow rate of 1.0 mL/min gave an optimal signal to noise ratio with a reasonable separation time. The overlain spectra of lamivudine, zidovudine and nevirapine in mobile phase showed isobestic point at 265 nm. Hence the detection wavelength was selected at 265 nm. Using reverse phase C_{18} column, the retention time for lamivudine, zidovudine and nevirapine were 3.36, 5.30 and 9.28 min, respectively. The total time of analysis was less than 15 min. By plotting peak area against their respective concentrations the linearity of lamivudine, zidovudine and nevirapine were found to be in the range of 36-84, 72-168 and 48-112 µg/mL, respectively. The corresponding values were depicted in Table-1.

TABLE-1
LINEARITY OF LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE

Conc. of lamivudine (µg/mL)	Peak area of lamivudine	Conc. of zidovudine (µg/mL)	Peak area of zidovudine	Conc. of nevirapine (µg/mL)	Peak area of nevirapine
36	1452646	72	2656640	48	1802599
48	1919333	96	3484531	64	2377344
60	2379463	120	4242959	80	2956095
72	2838108	144	5014538	96	3551175
84	3290569	168	5735451	112	4107292

The proposed method was successfully applied for the analysis of lamivudine, zidovudine and nevirapine labeled to contain lamivudine 150 mg, zidovudine 300 mg and nevirapine 200 mg as active substances. The results and statistical parameters were shown in Table-2. The low values of RSD % indicated high precision of the method. The precision of the method was demonstrated by repeatability studies. The precision of the proposed method was determined by assaying the standard solutions on the same day and on three different days over a period of two weeks as reproducibility and ruggedness, which were expressed in terms RSD %. The intra-day and inter-day precision has been depicted in Table-3. To confirm the accuracy of the proposed method, recovery experiments were carried out by

TABLE-2
STATISTICAL VALIDATION

Drug	Label claim (mg/tablet)	Amount estimated (mg/tablet)*	Amount estimated* (%)	RSD (%)
Lamivudine	150	150.60 ± 0.46	100.40 ± 0.31	0.31
Zidovudine	300	298.11 ± 0.89	99.37 ± 0.30	0.30
Nevirapine	200	199.10 ± 0.52	99.55 ± 0.26	0.26

*Mean of five determinations; RSD = Relative standard deviation.

TABLE-3
INTRA-DAY AND INTER-DAY PRECISION OF LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE

Drug	Theoretical concentration (µg/mL)	Intra-day measured concentration*		Inter-day measured concentration*	
		Mean of peak area*	RSD (%)	Mean of peak area*	RSD (%)
Lamivudine	48	1918560.83	0.07	1921735.47	0.31
	60	2382245.00	0.09	2411695.13	0.45
	72	2851905.00	0.23	2795283.05	0.95
Zidovudine	96	3460376.67	0.25	3435453.78	0.35
	120	4246337.33	0.15	4275341.19	0.28
	144	5038588.00	0.41	5052433.27	0.52
Nevirapine	64	2387223.17	0.22	2405317.25	0.47
	80	2970764.00	0.45	3012586.00	0.82
	96	3556472.50	0.06	3513254.50	0.17

standard addition technique by adding a known amount of standard at three different levels to the pre-analyzed sample. Each level was repeated three times and the amount of drug found by the assay method, results and statistical parameters are reported in Table-4.

TABLE-4
RECOVERY STUDIES OF LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE

Label claim (mg/tablet)	Sample conc. (µg)	Amount added (µg)	Amount recovered* (µg)	Recovery* (%)	Average recovery (%)	RSD (%)
Lamivudine 150 mg	36	7.5	7.42 ± 0.21	98.99	99.30	0.23
		15.0	14.87 ± 0.24	99.14		
		22.5	22.45 ± 0.24	99.78		
Zidovudine 300 mg	72	15.0	14.91 ± 0.39	99.40	99.58	0.41
		30.0	29.84 ± 0.41	99.47		
		45.0	44.95 ± 0.43	99.88		
Nevirapine 200 mg	48	10.0	9.86 ± 0.42	98.60	99.46	0.45
		20.0	19.90 ± 0.44	99.50		
		30.0	29.92 ± 0.49	99.75		

*Mean ± and standard deviation for five determinations.

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