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First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Lamivudine, Nevirapine and Zidovudine in Tablets

VAISHALI NAGULWAR* and KISHOR BHUSARI
Department of Pharmaceutical Chemistry, Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur-441 110, India
Tel: (91)(7104)232169; E-mail: vaishalinagulwar@yahoo.com

A simple, accurate, precise, economical and reproducible first order derivative UV spectrophotometric method is being developed for the simultaneous estimation of lamivudine, nevirapine and zidovudine in pure bulk drug and in tablet dosage form. The stock solutions were prepared in 0.5 M HCl followed by the further required dilutions with distilled water. The peak amplitude for lamivudine, nevirapine and zidovudine were observed at 266.70, 320.31 and 280.38 nm, respectively and linearity was also shown at these wavelengths in concentration range of 5-25, 10-50 and 10-40 µg/mL by all the 3 drugs. The proposed method has estimated lamivudine 98.5 %, nevirapine 99.89 % and zidovudine 99.72 % in marketed tablets. The results of analysis have been validated statistically and also by recovery studies.

Key Words: Spectrophotometry, Lamivudine, Nevirapine, Zidovudine.

INTRODUCTION

Lamivudine, nevirapine and zidovudine are anti HIV drugs. Out of the various classes of antiretroviral, lamivudine is a nucleoside analogue. Several methods including spectrophotometric[1], HPLC[2] and HPTLC[3] methods have been reported for its estimation in various dosage forms with other drugs. Nevirapine is a non-nucleoside reverse transcriptase inhibitor. The HPLC[4], HPTLC[5] and spectrophotometric[6] methods has been found in literature for its estimation alone or in fixed dose combination with other drugs. Like lamivudine, zidovudine is also a nucleoside analogue. Many methods have been reported for its estimation using spectrophotometry[7], HPLC[8,9] techniques. But no method has been found for the simultaneous estimation of selected fixed dose combination by first derivative UV spectrophotometric technique.

EXPERIMENTAL

Shimadzu 1601UV-visible spectrophotometer with a matched pair of 10 mm quartz cells was used. Lamivudine, nevirapine and zidovudine pure drugs (Cipla Ltd. Goa and Patalganga, India), hydrochloric acid (Loba, India Ltd.) and distilled water was used in the present study. The commercially available tablets containing a combination of lamivudine-150 mg, nevirapine-200 mg and zidovudine- 300 mg were procured from local pharmacy.

Preparation of standard stock solution: 1 mg/mL stock solutions of the drugs were prepared in 0.5 M HCl. For the linearity study, aliquots of the drug solutions were further diluted with distilled water to get the final working standards of concentration range as lamivudine 5-25 μg/mL, nevirapine 10-50 μg/mL and zidovudine 10-40 μg/mL, respectively.

The peak amplitude of the obtained first derivative spectra was measured at 266.70, 320.31 and 280.38 nm for lamivudine, nevirapine and zidovudine, respectively. Calibration graphs of all the three drugs were plotted at 266.70, 320.31 and 280.38 nm (Figs. 1-3). Upon examining the first derivative spectra of the three drugs (Fig. 4), it can be noticed that lamivudine can be determined at 266.70 nm where nevirapine and zidovudine has no contribution. Similarly nevirapine can be determined at 320.31 nm where lamivudine and zidovudine does not have any absorbance. Zidovudine can also be estimated at 280.38 nm where lamivudine and nevirapine have no interference.

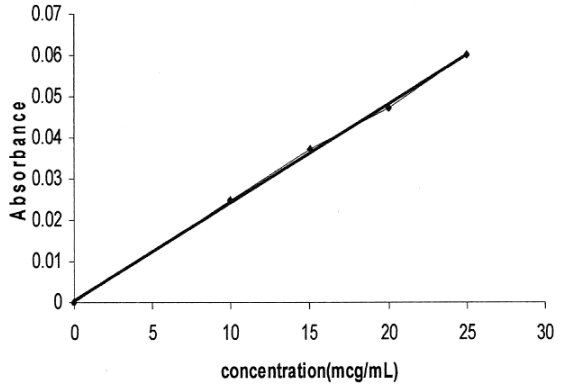

Fig. 1. Calibration curve for lamivudine at 266.70 nm

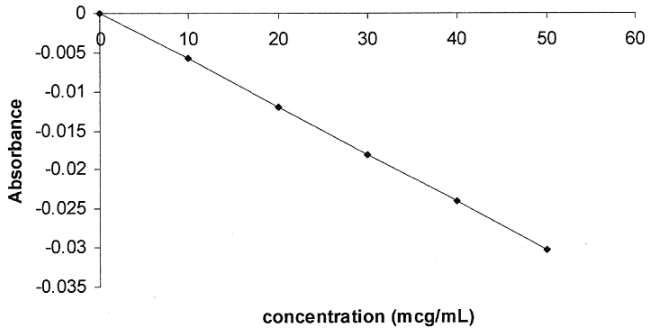

Fig. 2. Calibration curve for nevirapine at 320.31 nm

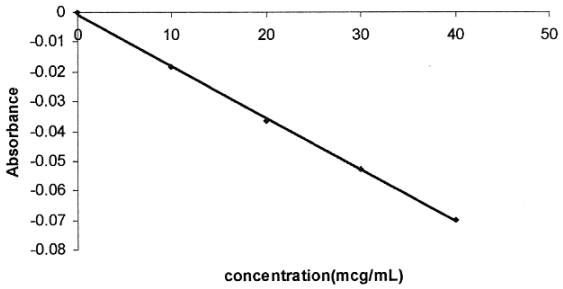

Fig. 3. Calibration curve for zidovudine at 280.38 nm

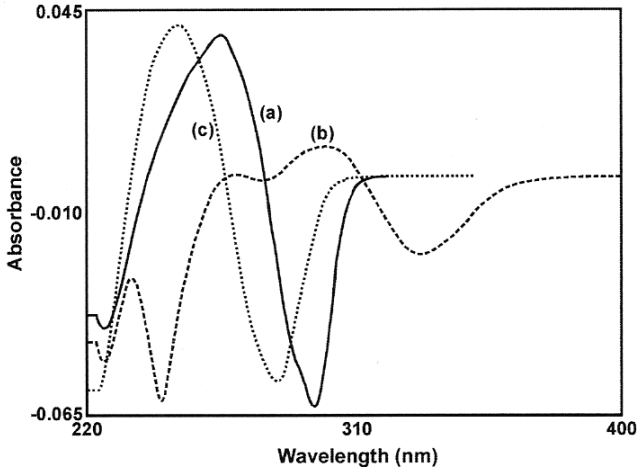

Fig. 4. First order derivative overlain spectra of lamivudine (a), nevirapine (b), zidovudine (c)

Lamivudine - A = 0.000448649 + 0.002380 × C, r = 0.999344608
Nevirapine - A = 0.000180952 + -0.000608 × C, r = -0.999903766
Zidovudine - A = -0.402218775 + -0.001744 × C, r = -0.999739707

where C is the concentration in mcg/mL, A is the peak amplitude of the first derivative curves at 266.70, 320.31and 280.38 nm for lamivudine, nevirapine and zidovudine, respectively and r is the correlation coefficient.

The proposed method was applied for the determination of lamivudine, nevirapine and zidovudine in the marketed tablets (Table-1). The accuracy, selectivity and validity of the method was further assessed by applying the standard addition technique in the recovery studies, wherein sample was spiked with known quantity of standard drug of lamivudine, nevirapine and zidovudine (Table-1). The percentage recovery was found to be lamivudine-100.03 % ± 0.02, nevirapine-100.03 % ± 0.02 and zidovudine-100.08 % ± 0.06 respectively. Validation of the findings was done appropriately and is represented in Table-2.

TABLE-1
RESULTS OF THE ANALYSIS OF COMMERCIAL FORMULATIONS AND THE RECOVERY STUDIES

Standard mixture	Concentration (µg/mL)	Content estimated (µg/mL)	% Amount estimated	Standard deviation (±)	–
Lamivudine	15	14.960	99.70	0.23	–
Nevirapine	20	20.012	100.06	0.04	–
Zidovudine	30	30.030	100.10	0.07	–
Tablet	Label claim (mg/tab)	Amount found (mg/tab)	% Label claim	Standard deviation (±)	% Recovery (± SD)
Lamivudine	150	147.75	98.50	1.06	100.03 ± 0.02
Nevirapine	200	199.78	99.89	0.08	100.12 ± 0.08
Zidovudine	300	299.16	99.72	0.12	100.08 ± 0.06

All the values are the mean of 5 readings.

TABLE-2
REGRESSION ANALYSIS OF CALIBRATION CURVES AND SUMMARY OF VALIDATION PARAMETERS

Parameters	Lamivudine	Nevirapine	Zidovudine
Wavelength (nm)	266.70	320.31	280.38
Beer's law limit (µg/mL)	5-25	10-50	10-40
Limit of detection (µg/mL)	1	5	1
Limit of quantitation (µg/mL)	1	10	5
Regression equation*			
Intercept (α)	0.000448649	0.000180952	-0.402218775
Slope (β)	0.002380811	-0.000608571	-0.001744000
Correlation coefficient (r)	0.999344608	-0.999903766	-0.999739707

Where, *$y = \alpha + \beta x$, x is the concentration of the analyte and y is the absorbance value.

RESULTS AND DISCUSSION

Thus, the proposed method has estimated the per cent amount of the pure drugs in standard mixture as lamivudine-99.70, nevirapine-100.06 and zidovudine- 100.1. The marketed tablet formulation has also been analyzed and the per cent of the label claimed were lamivudine-98.5, nevirapine-99.89 and zidovudine-99.72. The recovery studies done by standard addition method has given satisfactory results as lamivudine-100.03 % ± 0.02, nevirapine-100.3 % ± 0.08 and zidovudine-100.08 % ± 0.06, respectively.

The main advantage of the proposed method is its suitability for routine determination of lamivudine, nevirapine and zidovudine from their marketed tablet formulations. The proposed method is economic, simple, sensitive, precise and reproducible and do not require any expensive or sophisticated apparatus, in contrast with the reported chromatographic methods.

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