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Original article

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Abstract:

In this study an HPTLC-densitometric method was developed for the simultaneous determination of lamivudine, nevirapine and zidovudine in pharmaceutical dosage forms which are used widely as first line HIV/AIDS treatment. Chromatographic separation of the drugs was performed on precoated silica gel $60F_{254}$ (0.2 mm thickness) plates using n-hexane: chloroform: methanol (1:7:2, v/v) as a mobile phase. A TLC scanner set at 275 nm was used for the direct evaluation of the chromatogram in reflectance-absorbance mode. The three drugs were satisfactorily resolved with R_f values of 0.22 ± 0.03, 0.55 ± 0.03 and 0.73 ± 0.03 for lamivudine, zidovudine and nevirapine, respectively. Calibration curves were polynomial in the range 100-1300 ng/band for both lamivudine and nevirapine, and from 100-1700 ng/band for zidovudine. Correlation coefficients (r) values were 0.9999 for lamivudine and 0.9998 for nevirapine and zidovudine. A low relative standard deviation (< 2%) was found for both precision and robustness study showing that the proposed method was precise and robust. The method had an accuracy of 98.93%, 99.45% and 99.21%; and percentage assay of 99.91%, 98.72% and 99.34% for lamivudine, nevirapine and zidovudine, respectively. Method had the potential to determine these drugs simultaneously from dosage forms without any interference.

Keywords: Densitometric detection; HPTLC; Lamivudine; Nevirapine; Zidovudine; Simultaneous determination

Introduction

HIV/AIDS infection represents global health hazards and has enormous social, economical and ethical ramifications [1,2]. Today, HIV/AIDS is the leading cause of death in sub-Saharan Africa and the fourth biggest killer in the world [3]. Since the beginning of the epidemic, approximately 25 million people have died worldwide, making it one of the most devastating epidemics of all times. Currently, an estimated 33 million individuals are living with HIV-1 [4].

Up to 2008, exactly 25 anti-HIV compounds have been formally approved for clinical use in the treatment of AIDS. However, new anti-HIV agents are still needed to confront the emergence of drug resistance and various adverse effects associated with long-term use of antiretroviral therapy [5]. The current common treatment strategy for HIV-infected patients by highly active antiretroviral therapy involves multi-therapy drugs that contain two nucleoside reverse transcriptase inhibitors combined with one non-nucleoside reverse transcriptase inhibitor or one protease inhibitor or both [6]. Amongst these fixed dose combinations, lamivudine, nevirapine and zidovudine constitute first line therapy [7].

Lamivudine, 4-amino-1-[(*2R,5S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-pyrimidin-2(*1H*)-one (Fig. 1a), is a synthetic nucleoside analogue that is phosphorylated intracellularly to its active 5′-triphosphate metabolite [8]. Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2′,3′-f][1,4]-diazepin-6-one (Fig. 1b), is a non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type-1 [9]. It binds directly and non-competitively to allosteric-site on reverse transcriptase [10]. Zidovudine, 1-[(*2R,4S,5S*)-4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-pyrimidine-2, 4-dione (Fig. 1c), is the first nucleoside analogue approved by FDA [7]. It is phosphorylated intracellularly to its active 5′-triphosphate metabolite [8].

HPTLC is rapidly becoming a routine analytical technique in many product development and analytical laboratories due to its advantages [11]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase [12]. This reduces analysis time, cost per analysis and possibilities of pollution of the environment. Simultaneous assay of several components in a multi-component formulation is also possible [13,14].

So far no official analytical methods have been reported in British (2004), European (2008) [15] and United States (2009) [16] pharmacopoeias for the simultaneous quantifications of lamivudine, zidovudine and nevirapine in pharmaceutical combinations, but a few HPLC methods have been reported for the simultaneous determination of lamivudine, nevirapine and zidovudine mixtures in both pharmaceutical formulations [17,18] and biological fluids [7,19,20]. HPLC-MS-MS method for simultaneous determination of lamivudine, zidovudine and nevirapine with other anti-HIV drugs in human plasma has also been described

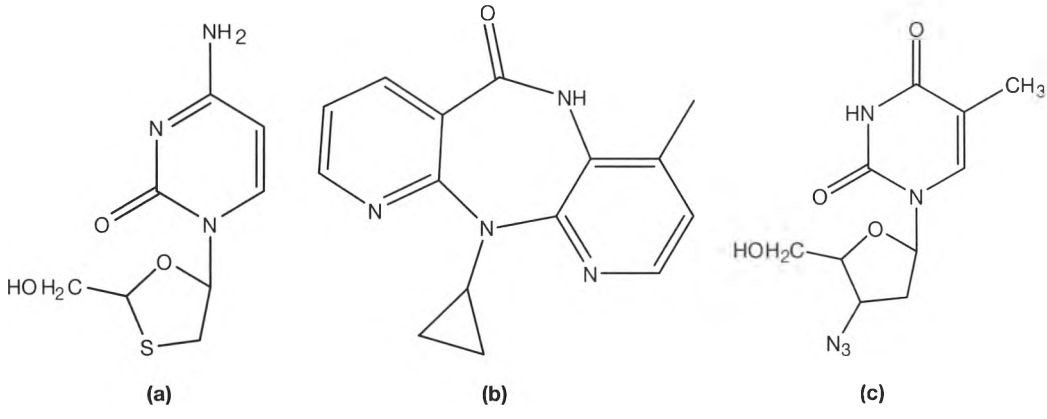

Figure 1 Chemical structures of lamivudine (a), nevirapine (b) and zidovudine (c)

(Jung *et al.*, 2007). As far as individual drug is considered, both Ph. Eur (2008) [15] and USP (2009) [16] recommend liquid chromatography and a number of reported HPLC methods are also available for the determination of lamivudine [21], nevirapine [22,23] and zidovudine [23,24]. Besides, a few HPTLC methods were described for the determination of lamivudine and zidovudine [25], lamivudine and nevirapine [26] and nevirapine [10].

The objective of this work was to develop and validate an accurate, precise, and simple HPTLC method for the simultaneous determinations of lamivudine, nevirapine and zidovudine combination in pharmaceutical formulations.

Experimental

Materials

Lamivudine, zidovudine and nevirapine working reference standards (Matrix Laboratories Ltd, India) were obtained from DACA. The dosage form, fixed dose combination tablet Zidolam-N® (Hetero Drugs Limited, India) with claimed labels of lamivudine 150 mg, nevirapine 200 mg and zidovudine 300 mg, was obtained from Yekatit 12 Hospital. HPLC grade methanol (BDH, England), analytical grade chloroform (Sigma-Aldrich, Germany), analytical grade n-hexane (BDH, England) were also used during the method development.

Instrumentation

The method development was done by using Computerized Camag HPTLC system (Camag, Muttenz, Switzerland) consisting of a Camag Linomat V semiautomatic spotting device connected to a nitrogen tank and WinCATS version 1.4.0 software (Camag, Muttenz, Switzerland), a Camag TLC scanner III (Camag, Muttenz, Switzerland) densitometer driven by the same WinCATS software, a Camag 100 µl HPTLC sample syringe (Hamilton, Bonaduz, Switzerland), a Camag glass twin-trough development chamber (20 cm x 10 cm; Camag, Muttenz, Switzerland), UV chamber (Camag, Muttenz, Switzerland), aluminum backed HPTLC plates (silica gel60 F_{254} 20 x 20 cm with 0.2 mm thickness, Camag, Muttenz, Switzerland) and 0.45 µm nylon syringe filters.

Standard solution preparation

Stock standard solution was prepared by dissolving 75 mg of lamivudine, 100 mg of nevirapine and 150 mg of zidovudine in 100 ml of methanol with the aid of ultrasonication for 5 min to get a concentration of 0.75 mg/ml, 1.0 mg/ml and 1.5 mg/ml for lamivudine, nevirapine and zidovudine, respectively. Then working standard solution was prepared by diluting 20 ml of the stock standard solution to a 100 ml volumetric flask using methanol as a diluent to give concentrations of 150 ng/µl, 200 ng/µl and 300 ng/µl for lamivudine, nevirapine and zidovudine, respectively.

Sample solution preparation

Twenty tablets (Zidolam-N®: containing 150 mg of lamivudine, 200 mg of nevirapine and 300 mg of zidovudine) were accurately weighed and powdered. A portion of the powder equivalent to 60 mg of lamivudine, 80 mg of nevirapine and 120 mg of zidovudine was weighed and quantitatively transferred in to a 100 ml volumetric flask. The powder was dissolved using methanol with the aid of mechanical shaking for 5 min and sonication for 15-20 min. The volume was then made to the mark with methanol and filtered to obtain a concentration of 0.6 mg/ml, 0.8 mg/ml and 1.2 mg/ml for lamivudine, nevirapine and zidovudine, respectively. Working sample solution was freshly prepared by taking an aliquot of 25 ml of the stock sample solution to a 100 ml volumetric flask and diluted with methanol to the mark to give a concentration of 150 ng/µl, 200 ng/µl and 300 ng/µl for lamivudine, nevirapine and zidovudine, respectively.

Chromatographic conditions

HPTLC was performed on 10 cm x 10 cm and 10 cm x 20 cm aluminum plates coated with 0.2 mm layers of silica gel 60F_{254}. Samples were applied to the plate 10 mm from the bottom and 10 mm from the side edges of the plate as 6.0 mm bands. The band was dried with the aid of an online nitrogen gas. Linear ascending development with n-hexane: chloroform: methanol (1:7:2, v/v/v) as mobile phase was performed in a Camag twin-trough glass chamber previously

saturated with mobile phase for 25 min at room temperature (25 °C). Approximately, 15 ml of mobile phase was used for each development. The optimized development distance and development time were 70 mm and 15 min respectively. After development the plates were dried in a current of hot air using a hair-dryer. Densitometric scanning was performed on Camag TLC scanner III in the reflectance mode at 275 nm and operated by winCATS software for quantification. The slit dimension of the scanner was set at 5 mm x 0.30 mm and 20 mm/s scanning speed was employed.

Method Validation

The method was validated in accordance with ICH guidelines [27] for the following parameters.

Linearity

Linearity of the peak area response was determined by making five measurements at seven concentration points in the range of 100-1300 ng/band for lamivudine and nevirapine and five concentration points in the range of 100-1700 ng/band for zidovudine.

Precision

To study repeatability of sample application and measurement of the peak area, six determinations at a concentration of 300 ng/band for lamivudine, 400 ng/band for nevirapine and 600 ng/band zidovudine were made. Repeatability of sample application and measurement of the peak area were evaluated by comparing the relative standard deviations obtained from the peak area measurements. To study the precision of the method, both intra-day and inter-day precision were applied. Intra-day precision was studied by taking three different concentrations 300, 450 and 600 ng/band of lamivudine, 400, 600 and 800 ng/band of nevirapine and 600, 900 and 1200 ng/band of zidovudine. The given concentrations were applied three times to see the variation of their peak area with in a day. For inter-day precision the same concentrations were applied but their peak area variation was studied for three different days.

Accuracy/recovery studies

The recovery study was carried out by applying the proposed method on the drug sample to which known amount of lamivudine, nevirapine and zidovudine standard had been added (standard addition method). Four determinations were done to study the recovery. To study the recovery of lamivudine 50, 100, 150 and 200 mg of lamivudine standard were added to the labeled claim of lamivudine 150 mg (i.e. the spiked amounts were 400, 500, 600 and 700 ng/band). To study the recovery of nevirapine 100, 150, 200 and 250 mg of nevirapine standard were added to the labeled claim of nevirapine 200 mg (i.e. the spiked amounts were 600, 700, 800 and 900 ng/band). Similarly, for zidovudine recovery study 200, 250, 300 and 350 mg of zidovudine standard were added to the labeled claim of zidovudine 300 mg (i.e. the spiked amounts were 1000, 1100, 1200 and 1300 ng/band). Finally, the % recovery of lamivudine, zidovudine and nevirapine was compared with the actual amounts.

Limit of detection and quantification

Detection and quantification limits (LOD and LOQ) were calculated from the calibration equations obtained from the experiment. Determinations of detection and quantification limits were based on the standard deviation of the response and the slope as:

$$LOD = 3.3\sigma/S, \text{ and } LOQ = 10\sigma/S \quad (1)$$

Where σ is the standard deviation of y-intercepts of regression line and S is the slope of the corresponding standard curve.

Peak purity test/specificity

The spots of the samples were confirmed by comparing the R_f values and spectra of the spots with that of the standards. Moreover, the peak purity of samples was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot [28, 29].

Robustness

To study the robustness of the method, slight but deliberate changes were made in some parameters. Parameters such as the mobile phase composition, total mobile phase volume, time from application to development and time from development to scanning were used to study the robustness at 10% variation.

Concentrations of 300 ng/band for lamivudine, 400 ng/band for nevirapine and 600 ng/band for zidovudine were applied to study the robustness of the method. The effect of these changes on R_f values and peak area was evaluated by calculating the relative standard deviations (RSD) for each parameter.

Results and Discussion

Method optimization for the HPTLC-densitometric measurements

Selection of appropriate mobile phase is generally done by controlled trial and error based on the analyst's experience and search of literature [30]. Various solvent systems were evaluated to arrive at an optimum resolution of the three drugs. A combination of two solvents, one non polar and the other relatively polar was not enough to separate the two more polar drugs i.e. lamivudine and zidovudine. Thus it was necessary to use a third solvent to separate the two more polar drugs. After a number of trials had been made by changing both the polarity and composition of different solvents, n-hexane: chloroform: methanol in a 1:7:2 (v/v) gave well separated, compact spots as well as sharp peaks for the drugs in the mixture. The R_f values were 0.22, 0.55 and 0.73 for lamivudine, zidovudine and nevirapine, respectively which were suitable for quantification.

Fig. 2 shows the HPTLC densitogram of the three drugs using the optimal conditions stated previously. After chromatographic development, bands were scanned over the range of 200-400 nm. The λ max for lamivudine, zidovudine and nevirapine were at 276, 270 and 289 nm, respectively. Quantitative determinations of lamivudine, nevirapine and zidovudine were performed by scanning the spots at 275 nm in absorbance/reflectance mode (Fig. 3).

Method validation results

Linearity and calibration curves

Calibration graphs for the three drugs were constructed by plotting peak areas against the corresponding concentrations (ng/band). According to ICH guidelines for validation of analytical methods, the linear function is required [31]. Therefore both linear and polynomial regressions were tested. The linear relationship was found to be less precise due to the minimal fitting of the residuals on the calibration line indicating lower precise correlations (r = 0.9907, 0.9933 and 0.9918 and r^2 = 0.9815, 0.9867 and 0.9836

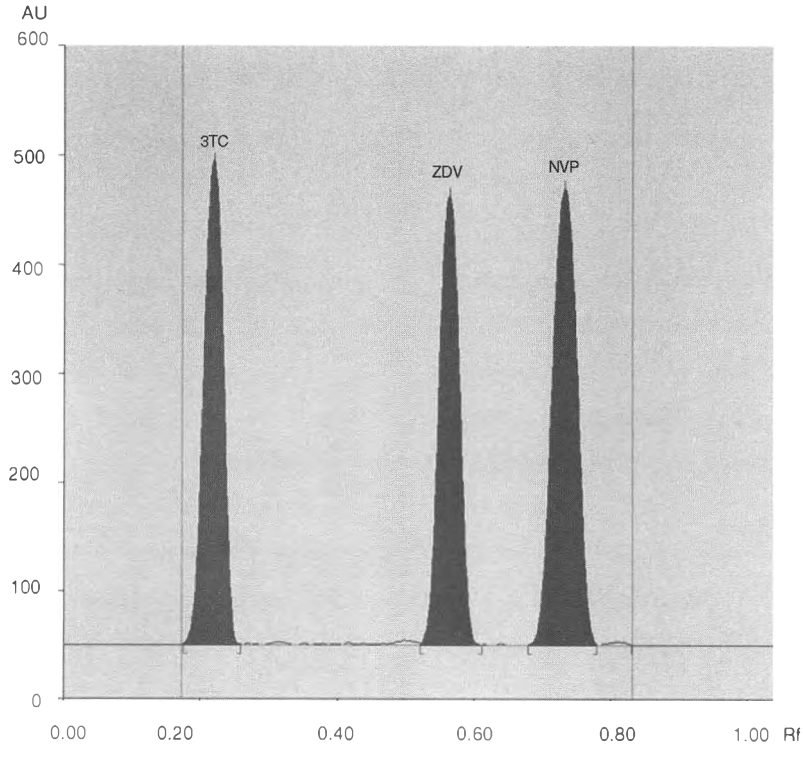

Figure 2 HPTLC densitogram of lamivudine (3TC), nevirapine (NVP) and zidovudine (ZDV) all at 900 ng/band

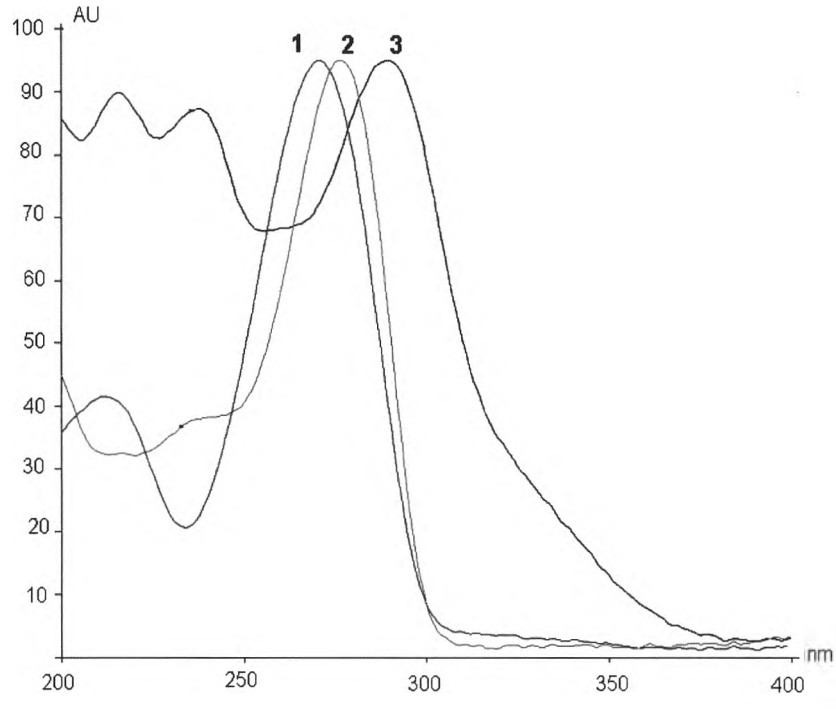

Figure 3 Typical UV-spectra of zidovudine (1), lamivudine (2) and nevirapine (3)

Table 1 Characteristic parameters for the polynomial regression equation (n=5)

Parameters	Lamivudine	Nevirapine	Zidovudine
Linearity range (ng/µl)	100-1300	100-1300	100-1700
Coefficient of X^2 ± *S.D*	-0.0030 ± 0.0034	0.0031 ± 4.47E-05	0.002
*Coefficient of X ± *S.D*	11.986 ± 0.095	13.576 ± 0.0518	10.898 ± 0.034
Intercept ± *S.D*	623.94 ± 15.83	288.22 ± 54.62	440.67 ± 12.15
Determination coefficient r^2 ± *S.D*	0.9998 ± 4.47E-05	0.9997 ± 0.00012	0.9996 ± 0.0016
Correlation coefficient r	0.9999	0.9998	0.9998

*Coefficient of X = slope of the graph

for lamivudine, nevirapine and zidovudine, respectively). The second order polynomial fit was found to be more suitable and its residuals plot showed a much better fitting than that of linear model, indicating good correlation (r = 0.9999, 0.9998 and 0.9998 and r^2 = 0.9998, 0.9997 and 0.9996 for lamivudine, nevirapine and zidovudine, respectively). The calibration graphs were constructed in the range of 100-1300 ng/band for lamivudine and nevirapine; and 100-1700 ng/band for zidovudine. This method showed that a wider linearity (range) than the methods reported by Kaul and his co-workers 300-1000 ng/band for nevirapine [10]; and Girum and his co-workers 250-1400 and 250-1700 ng/band for lamivudine and zidovudine, respectively [25]. The characteristic parameters of the second order polynomial regression equation of the three drugs are shown in Table 1.

Precision

Repeatability of sample application and measurement of peak area were expressed in terms of RSD [28]. Data obtained from precision experiments for repeatability studies are given in Table 2. Since the RSD obtained in all cases is less than 2.0%, it is possible to conclude that the instrument has good precision [25]. Intra-day precision was determined by analysis of standard solutions three times on the same day while

Table 2 System precision studies of the developed method

	Sample application			Measurement of area		
	Lamivudine (300 ng/µl)	Nevirapine 400 (ng/µl)	Zidovudine (600 ng/µl)	Lamivudine (300 ng/µl)	Nevirapine 400 ng/µl)	Zidovudine (600 ng/µl)
	4023.68	5278.89	6193.99	4057.43	5251.13	6243.01
	3986.90	5180.31	6256.82	4045.13	5269.98	6259.19
	3978.62	5266.04	6268.02	3997.24	5246.15	6250.32
	4017.46	5190.02	6195.41	3989.65	5191.29	6202.11
	4001.25	5242.86	6249.73	3985.35	5182.04	6198.71
	3987.82	5196.44	6208.71	3991.29	5181.79	6187.12
Mean	3999.29	5225.76	6228.78	4011.02	5220.40	6223.41
R.S.D.	0.45	0.81	0.53	0.79	0.76	0.50

Table 3 Method precision studies of the developed method

Intra-day precision (n=3)			Inter-day precision (n=3)					
			Day 1		Day 2		Day 3	
Amount (ng/band)	Mean area (AU)	RSD	Mean area (AU)	RSD	Mean area (AU)	RSD	Mean area (AU)	RSD
Lamivudine								
300	4013.99	1.08	4013.99	1.08	4005.08	0.79	4023.25	0.87
450	5413.50	0.66	5413.50	0.66	5414.70	0.48	5447.98	0.93
600	6712.87	0.60	6712.87	0.60	6718.35	0.63	6703.36	0.56
Nevirapine								
400	5225.39	0.77	5225.39	0.77	5213.87	0.61	5234.29	0.86
600	7356.46	0.58	7356.46	0.58	7333.85	0.57	7354.74	0.93
800	9210.32	0.36	9210.32	0.36	9252.23	0.58	9209.27	0.36
Zidovudine								
600	6179.19	1.05	6179.19	1.05	6248.17	0.80	6232.78	0.67
900	8629.18	0.48	8629.18	0.48	8642.65	0.38	8685.01	0.63
1200	10682.55	0.23	10682.55	0.23	10656.48	0.86	10636.07	0.43

inter-day precision was determined by analysis of similar standards on three different days over a period of one week [32]. The data in Table 3 shows the intra and inter-day variation for the determination of lamivudine, nevirapine and zidovudine at three different concentration levels. Relative standard deviation was calculated for both series of analyses and the results show that the method is precise.

Accuracy/recovery studies

To check for the accuracy of the proposed method, recovery experiments were carried out by standard addition technique by adding a known amount of standard at four different levels to the pre-analyzed sample; and analysis of each level was repeated three times ($n = 3$) [33]. As indicated in Table 4, good recoveries of the product in the range of 98.85 to 99.90%, 98.20 to 99.83% and 98.51 to 99.21% were obtained for lamivudine, nevirapine and zidovudine respectively, which indicated high accuracy of the method.

Limit of detection and quantification

The limit of detection and limit of quantification for the proposed method which were calculated

Table 4 Recovery study for determinations of lamivudine, nevirapine and zidovudine

Amount of standard drug added (%)		Amount of drug analyzed (ng)	Theoretical concentration (ng)	Amount recovered (ng)	% recovery	RSD
Lamivudine	33.33	300	400	395.40	98.85	0.45
	66.67	300	500	497.49	99.50	0.75
	100.00	300	600	599.60	99.90	0.71
	133.33	300	700	694.70	99.24	0.74
Nevirapine	50	400	600	589.20	98.20	0.38
	75	400	700	698.80	99.83	0.23
	100	400	800	790.30	98.79	0.65
	125	400	900	885.50	98.33	0.36
Zidovudine	66.66	600	1000	985.10	98.51	0.47
	83.33	600	1100	1089.75	99.07	0.25
	100.00	600	1200	1190.50	99.21	1.90
	116.67	600	1300	1280.00	98.46	0.74

Table 5 Data for robustness testing of the developed method (n=3)

Parameters	Lamivudine		Nevirapine		Zidovudine	
	Mean area (AU)	RSD	Mean area (AU)	RSD	Mean area (AU)	RSD
Mobile phase composion	4030.05	1.67	5228.00	1.19	6200.69	1.60
Amount of mobile phase	4004.74	0.94	5246.48	0.78	6224.37	0.75
Time from spotting to development	4009.77	0.46	5217.00	0.31	6214.40	0.31
Time from development to scanning	4013.15	0.74	5236.87	0.70	6236.72	0.51

Table 6 The effect of mobile phase composition and volume variation on R_f values

Mobile phase composion (V/V)	R_f value		
(n-hexane: chloroform: methanol)	Lamivudine	Nevirapine	Zidovudine
1.5: 10.5: 3 (optimized)	0.22	0.73	0.55
1.7: 10.5: 3	0.21	0.70	0.55
1.3: 10.5: 3	0.23	0.74	0.57
1.5: 11.5: 3	0.19	0.70	0.51
1.5: 9.5: 3	0.25	0.71	0.56
1.5: 10.5: 3.3	0.26	0.74	0.59
1.5: 10.5: 2.7	0.19	0.70	0.51
Volume of mobile phase (ml)			
15 (optimized)	0.22	0.73	0.55
16.5	0.23	0.73	0.56
13.5	0.23	0.72	0.56

Table 7 Summary of method validation parameters

Parameters	Lamivudine	Nevirapine	Zidovudine
Linearity range(ng/spot)	100-1300	100-1300	100-1700
Determination coefficient, r^2	0.9998	0.9997	0.9996
Correlation coefficient, r	0.9999	0.9998	0.9998
Limit of detection (ng/band), LOD	4.36	13.28	3.68
Limit of quantification (ng/band), LOQ	13.21	40.23	11.15
Recovery (Mean ± SD)	99.37 ± 0.44	98.71 ± 0.84	98.89 ± 0.78
Precision (RSD)			
Repeatability of application (n=6)	0.45	0.53	0.76
Repeatability of measurement (n=6)	0.81	0.79	0.50
Intra-day* (n=3)	0.78	0.57	0.59
Inter-day** (n=3)	0.73	0.62	0.61
Robustness	Robust	Robust	Robust
Selectivity	Selective	Selective	Selective

*Mean of three concentrations in the same day; **Mean of three concentrations in 3 different days

according to 3.3σ/S and 10σ/S criteria respectively are shown in Table 7. The limit of detection for lamivudine, nevirapine and zidovudine was found to be 4.36, 13.28 and 3.68 ng/band, respectively. Similarly, the limit of quantification for lamivudine, nevirapine and zidovudine was 13.21, 40.23 and 11.15 ng/band, respectively. Accordingly, zidovudine has the lowest limit of detection and quantification. These results showed that the method has the same sensitivity for lamivudine and zidovudine with the works of Girum and his co-workers with limit of detection 3.06 and 3.34 ng/band; and limit of quantification 9.28 and 10.13 ng/band for lamivudine and zidovudine, respectively [25]. However, the limit of detection and quantification of nevirapine was found to be higher than the reported method by Kaul and his co-workers with 5 and 10 ng/band, respectively [10].

Peak purity test/specificity

The peak purity test of lamivudine, nevirapine and zidovudine spots were assessed by comparing their respective spectra at peak start (S), peak apex (A), and peak end (E) position of the spots. The peak purity was found to be 0.9999, 0.9999 and 0.9999 at peak start and peak apex ($r_{(S, M)}$); and 0.9991, 0.9998 and 0.9998 at peak apex and peak end ($r_{(M, E)}$) for lamivudine, nevirapine and zidovudine, respectively. Based on the results correlation, it can be concluded that excipients present in the formulation did not interfere with the peaks of the samples [34]. Good correlation (r = 0.9999, r = 9992 and r = 0.9999) was also obtained between standard and sample spectra of lamivudine, nevirapine and zidovudine, respectively.

Robustness

Robustness of the method was studied by determining the effects of small variations of mobile phase volume and composition (± 10%). The time from spotting to chromatography and from chromatography to scanning was also varied by ± 10 min [33]. The relative standard deviation of peak areas was calculated for each parameter and was found to be less than 2%. The low RSD values as shown in Table 5 indicate robustness of the method [11,28]. Though the method was robust at 10% variation of the chosen parameters; mobile phase composition variation was found to be a sensitive parameter during the optimization process. Besides, the R_f value of the drugs (Table 6) were robusted for 10% variation of the composition and volume of the mobile phase.

Analysis of the marketed formulation

The proposed method was applied to the determination of lamivudine, nevirapine and zidovudine in commercial fixed dose combination tablets (Zdolam-N®). The procedure was repeated five times, individually weighing the tablet powder each time [35]. The

Table 8 Assay results of the fixed dose combination tablets (n=5)

Parameters	Lamivudine	Nevirapine	Zidovudine
Commercial tablet (Zidolam-N®):			
Label claim (mg/tablet)	150	200	300
Actual amount added (ng/band)	300	400	600
Amount obtained (ng/band) ± SD	299.74 ± 5.81	394.88 ± 9.58	596.01 ± 8.05
Drug content (%) ± SD	99.91 ± 1.94	98.72 ± 2.21	99.34 ± 1.34
RSD	1.94	2.24	1.35

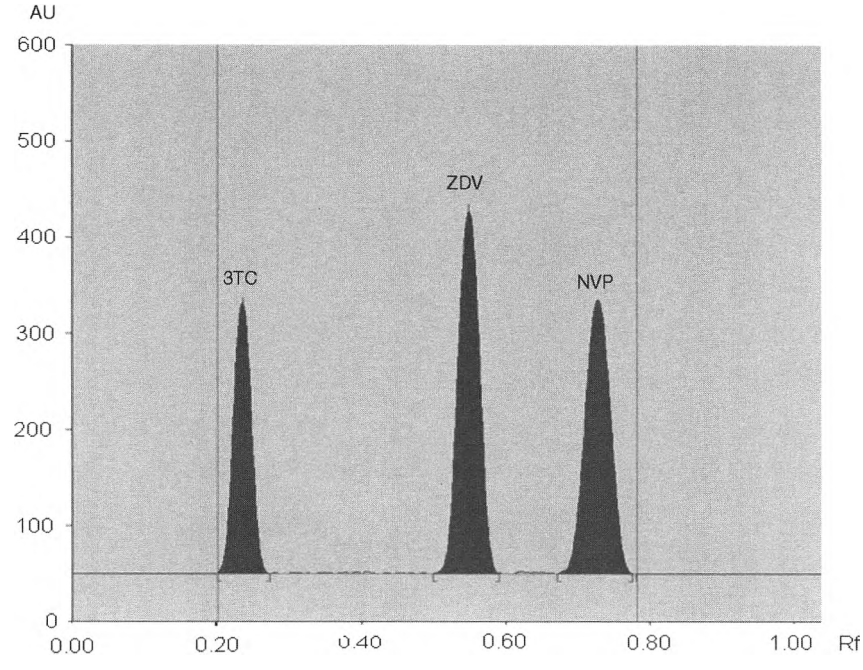

Figure 4 Typical HPTLC densitogram of lamivudine (3TC, 300 ng/band), nevirapine (NVP, 400 ng/band) and zidovudine (ZDV, 600 ng/band) of the dosage form

densitometric responses from the standard and sample were used to calculate the amounts of the drug in the tablet [36]. Assay results (Table 8) of lamivudine, nevirapine and zidovudine in tablets; expressed as a percentage of label claims were in good agreement with 90 to 110% of the label claims [16]. Moreover, only three peaks at R_f = 0.22, 0.55 and 0.73 were observed in the densitogram of the drug sample (Fig. 4), thereby suggesting that there was no interference from any of the excipients which are normally present in the tablets [29]. The low RSD showed that the method and the systems are suitable for routine analysis of the three compounds in pharmaceutical dosage form [28,36].

Stability in sample solution

The time the sample is left to stand in the solution prior to chromatographic development can influence the stability of drugss and are required to be investigated [28]. Solutions of different concentrations 300 ng/band for lamivudine, 400 ng/band for nevirapine and 600 ng/band for zidovudine were prepared and stored at room temperature for 0.5, 1.0, 2.0, 4.0 and 24.0 hours [25,28]. They were then applied on the HPTLC plate. After development, the densitogram was evaluated for the presence of additional spots if any. However, there was no any additional spot observed indicating the solutions stability during the analysis time. Furthermcre, Table 9 shows a low RSD value for stability testing indicating that the drugs in solution are stable for at least 24 hours.

Conclusion

In this work, simultaneous determination of lamivudine, nevirapine and zidovudine in fixed dose

Table 9 Stability of lamivudine, nevirapine and zidovudine in sample solutions (n=3)

Time (hr)	Amount (ng)	Peak area-1 (AU)	Peak area-2 (AU)	Peak area-3 (AU)	Mean area (AU) ± SD	RSD
Lamivudine						
0.5	300	3975.16	4020.20	3981.32	3992.23 ± 24.42	0.61
1		3993.81	4021.02	4003.12	4005.98 ± 13.83	0.35
2		3966.05	3976.73	4005.06	3995.95 ± 20.16	0.51
4		3966.44	3941.17	3991.96	3966.52 ± 25.40	0.64
24		3967.96	3894.06	3977.36	3946.46 ± 45.62	1.16
Nevirapine						
0.5	400	5179.83	5204.41	5166.39	5183.54 ± 19.28	0.37
1		5098.28	5194.11	5170.73	5154.37 ± 49.97	0.97
2		5198.28	5092.31	5184.12	5158.24 ± 57.53	1.12
4		5107.31	5099.59	5185.86	5130.92 ± 47.74	0.93
24		5093.55	5167.26	5055.56	5105.46 ± 56.79	1.11
Zidovudine						
0.5	600	6207.41	6242.65	6189.98	6213.35 ± 26.83	0.43
1		6195.62	6163.98	6233.56	6197.72 ± 34.83	0.56
2		6173.54	6199.97	6207.38	6193.63 ± 17.79	0.29
4		6199.77	6092.45	6167.97	6153.40 ± 55.12	0.90
24		6086.77	6191.12	6095.58	6124.49 ± 57.87	0.94

combination tablets, using HPTLC-densitometric method has been applied. The developed HPTLC method provides simple, precise and accurate analytical method for the simultaneous determinations of the three drugs in pharmaceutical formulations. The method was validated as per the ICH guidelines. Statistical analysis proved that the method provides a good recovery of the active ingredients that indicated the method's suitability to quantify the studied drugs in pharmaceutical formulations without any interference from excipients. In addition to simultaneous assay of several components in a multi-component formulation, the method utilized the merit of applying several samples on HPTLC plate that can minimize the cost of reagents and time for analysis. Hence, introducing an HPTLC-densitometric technique into pharmaceutical analysis represents a major step in terms of quality assurance laboratories particularly to facilitate the post marketing surveillance program.

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