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HPTLC-densitometry method development and validation for simultaneous determination of abacavir, lamivudine and zidovudine in combined dosage form

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Abstract

A simple, accurate and precise HPTLC- densitometry method for the simultaneous determination of abacavir, lamivudine and zidovudine in pharmaceutical tablet has been developed. Separation of tablet components was performed on aluminum backed HPTLC plates (silica gel 60 F_{254} 20×20 cm with 0.2 mm thickness, Camag, Muttenz, Switzerland) using methanol : chloroform : acetonitrile (4 : 8 : 3 v/v). Good sensitivity for all analytes was observed with UV detection at 275 nm. The method was validated for linearity, accuracy, precision, specificity and robustness. The method allowed quantitation over the 200–1450 ng/band range for the three components. Both linear and second order polynomial relationships were studied. 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 and determinations (r = 0.99995, 0.9998 and 0.9998 and r^2 = 0.9999, 0.9997 and 0.9996 for 3-TC, ABC and AZT, respectively). The method has an accuracy of 99.35, 99.19 and 99.13 % for 3-TC, ABC and AZT respectively. The method is robust and has the potential to determine these drug substances simultaneously from the dosage forms.

Keywords: HPTLC; Abacavir; Lamivudine; Zidovudine; Densitometry; Simultaneous; Determination.

1. Introduction

As the world enters the third decade of the AIDS epidemic, this pandemic has spread rapidly through the human population and became one of the leading causes of mortality worldwide [1]. For the dual purpose of increasing the effectiveness of antiviral therapy and preventing the

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development of a therapy-limiting viral resistance, the use of combination regimens of three or more antiretrovirals, often referred to as highly active antiretroviral therapy (HAART) has proven extremely effective in reducing the morbidity and mortality associated with HIV infection [2-4].

The high pill burden associated with the standard triple therapy regimens may eventually lead to non-adherence to treatment [5]. Combinations of drugs are necessary to treat HIV/AIDS successfully and there is renewed interest in fixed-dose combination products (FDCs) as a means of improving patient concordance and clinical outcomes. The WHO has endorsed the use of FDCs of antiretroviral drugs especially in developing countries [6].

TRIZIVIR tablets are for oral administration and each film-coated tablet contains 300 mg of ABC as abacavir sulfate, 150 mg of 3-TC, and 300 mg of AZT. ABC, 3-TC and AZT are all synthetic nucleoside analogues that get converted by cellular enzymes to the active metabolite and inhibit the activity of HIV-1 reverse transcriptase both by competing with the natural substrate triphosphates and *via* DNA chain termination after incorporation of the nucleotide analogue (Fig. 1) [5,7,8].

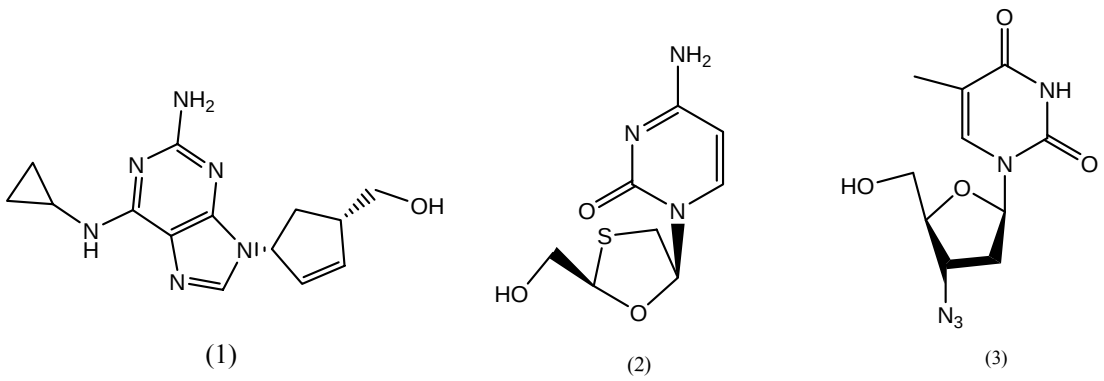

Fig. 1. Chemical structure of Abacavir (1), lamivudine (2) and zidovudine (3).

Although TLC is mainly used as a drug screening and confirmation tool, it has recently attracted considerable interest in quantitative pharmaceutical analysis due to improved technologies with HPTLC [9,10]. Modern instrumental HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks [11]. The HPTLC technique has been improved to incorporate features such as HPTLC grade stationary phase, automated sample application devices, automated developing chamber; computer controlled densitometry and quantitation, and fully validated procedures [9] and thus provides faster separation and better resolution [12].

A number of non official methods have been reported for the determination of AZT, 3-TC and ABC in dosage form and biological fluids when they are present as individual drugs or as a combination with each other or other antiretroviral drugs. There are several publications describing analytical methods for the determination of ABC, 3-TC and AZT individually. A few methods have been described for the simultaneous determination of ABC, 3-TC and AZT, as binary mixtures, in biological samples and pharmaceutical dosage forms [13-19]. Most of the reported methods require solid phase extraction or expensive equipment unsuitable for routine use in pharmacokinetic and pharmaceutical studies where many samples need to be analyzed.

So far no analytical method has been reported for simultaneous quantification of the ABC, 3-TC and AZT using HPTLC. However, some non HPTLC methods have been reported for the simultaneous determination of the three drugs or triple combination. Recently multivariate calibration methods have been used to resolve mixtures of two or more compounds with similar spectral characteristics [20, 21] and a chemometric optimization of a RP-HPLC for the simultaneous determination of ABC, 3-TC and AZT has been developed [22]. In addition to that a fully automated HPLC method for the simultaneous determination of ABC, 3-TC and AZT in

pharmaceutical tablets, human serum samples and drug dissolution studies has been developed [23]. ABC, 3-TC and AZT are quantified with other nucleoside/nucleotide reverse transcriptase inhibitors in human plasma by HPLC-MS-MS [24,25], HPLC –UV [26].

2. Material and Methods

2.1. Instruments

Computerized Camag HPTLC system (Camag, Muttenz, Switzerland), Micro syringe (Linomat syringe 695.0014, Hamilton-Bonaduz, Schweiz, Camag, Switzerland), twin trough chamber 20x10 cm (Camag, Muttenz, Switzerland), aluminum backed HPTLC plates (Silica gel60 F_{254} 20×20 cm with 0.2 mm thickness, Camag, Muttenz, Switzerland) and 0.45 µm nylon syringe filters were used during the study.

2.2. Chemicals and solvents

Working standards of 3-TC and ABC were obtained from Strides Arcolab limited, India, and AZT from Aurobindo Pharma Limited, India. Trizivir® Tablets (claimed labeled amount: 150 mg 3-TC, 300 mg abacavir sulphate and 300 mg AZT per tablet) manufactured by Glaxo Welcome Inc., HPLC grade methanol (Sigma-Aldrich, Germany), HPLC grade acetonitrile and chloroform (Fisher scientific, UK) were used.

2.3. Preparation of standard solutions

Stock solutions of the reference standards were prepared by transferring 50 mg of 3-TC, 100 mg of ABC and 100 mg of AZT in to a 100 ml volumetric flask and dissolving in about 80 ml of methanol with the aid of ultrasonication for 5 minutes. It was then diluted to volume with methanol to obtain a solution containing 0.5mg/ml, 1mg/ml and 1mg/ml of 3-TC, ABC and AZT, respectively. Working standard solution was prepared by diluting of 20 ml of the stock solutions to 100 ml using methanol to give a concentration of 200µg/ml for ABC and AZT and 100µg/ml for 3-TC. The final concentration ratio of ABC to 3-TC to AZT was made to be the same as that of the dosage form analyzed.

2.3.1. Preparation of sample solution (dosage form)

Twenty tablets containing 150 mg of 3-TC, 300 mg abacavir sulphate and 300 mg of AZT were accurately weighed and powdered. A portion of the powdered tablets equivalent to the label claim for one tablet was weighed accurately five times and each portion was transferred in to 100 ml volumetric flasks. The powder was mixed with 80 ml of methanol and after 15 minutes of mechanical shaking and 5 minutes of ultrasonication the solution was filtered through 0.45 µm syringe filter. Volume of the filtrate was adjusted to 100 ml with methanol to give a 3.00mg/ml of ABC and AZT and 1.5mg/ml of 3-TC. Working sample solution was prepared by dilution of 10 ml of the above sample stock solution to 100 ml with methanol to give a concentration of 300µg/ml of ABC and AZT and 150 µg/ml of 3-TC.

2.4. Chromatographic conditions

In optimization of the composition of the mobile phase, a number of trials were performed to get well resolved and compact spots for the three drugs from their mixture. The UV-Visible spectra of the spots of 3-TC, ABC and AZT were also obtained in the 200 to 400 nm range using a densitometer. The developed chromatograms were dried using hair dryer for 20 to 25 seconds and kept in UV cabinet for 5 minute before the start of the scanning at the selected wavelength using deuterium lamp. Reflectance/absorbance mode with slit width of 5x0.45 mm was

employed. Data resolution of 100μm/step, filter factor of Savitsky-Golay 7, with spectral scan speed of 100 nm/s and data resolution of 10nm/step and a scanning rate of 20 mm/s were used.

2.5. Validation of the developed method

2.5.1. Determination of linearity and range

Linear relationship between peak area or peak height of the spots and concentration of the drugs was evaluated by making five measurements over the concentration range of 200-1450 ng/band for all drugs. Six different concentration levels of calibration standard solutions were freshly prepared by serial dilution of 2-14.5 ml (with an interval of 2.5ml) of the stock standard solution of ABC and AZT and 4-29 ml (with an interval of 5ml) of the stock standard solution of 3-TC in appropriate volumetric flasks using methanol as diluent to get 200, 450, 700, 950, 1200, 1450 ng/μl respectively for the three drugs.

2.5.2. Precision studies

Precision of the method was checked by system and method precision parameters. System precision was conducted using the repeatability of sample application by making six measurements on a single stock solution (3-TC = 300.00 ng/band, ABC=600.00 ng/band and AZT = 600 ng/band) and repeatability of measurement of peak area was determined at the same concentration by scanning the developed spot six times with out changing the plate position.

Method repeatability was carried out by studying the intra-day and intermediate variation. The intra-day precision was performed by repeating the experiment six times in the same day. The intermediate precision was studied by considering inter-day precision and variation between analysts. For the inter-day precision the average of the data for the intra-day experiment was employed and two further experiments were performed in different days within a week. The relative standard deviation (RSD) at each level was calculated. To study analysts' variation, three determinations of the dosage form at 600.00 ng/band for both ABC and AZT and at 300.00 ng/band for 3-TC was carried out by three analysts. The intra-day and inter-day variation for determination of 3-TC, ABC and AZT was carried out at three different concentration levels of 300.00, 600.00, 1200.00 ng/band.

2.5.3. Accuracy/recovery studies

In order to examine the accuracy of the method and to check the interference from excipients used in the studied formulation, recovery studies were carried out by standard addition method. Recovery studies were carried out by applying the proposed method on the drug sample to which known amount of 3-TC, ABC and AZT standard corresponding to 80, 100 and 120 % of label claim had been added (standard addition method). At each level five determinations were performed and the results obtained were compared with the theoretical amounts.

2.5.4. Detection and quantification limits

The detection and quantification limits of the developed method were calculated using the following formulae [27,28]:

$$LOD = \frac{3.3\,\sigma}{S} \qquad (1)$$

$$LOQ = \frac{10\,\sigma}{S} \qquad (2)$$

Where σ = the standard deviation of the blank response, S = Sensitivity parameter

2.5.5. Peak purity test

The peak purity of drugs 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. Comparisons of spectra were performed automatically by win CATS version 1.4.0 software.

2.5.6. Robustness

Parameters used to evaluate robustness of the proposed method were composition and volume of the mobile phase, and time from spotting to development and from development to scanning. The composition of the mobile phase and the optimized volume of the mobile phase (15 ml) were altered by $\pm$10%. Time from spotting to development and from development to scanning was varied from the optimized time in the ranges of ± 20% and up to a 1 hour period. The effect of these changes on R_f values and peak area was evaluated by calculating the relative standard deviations (RSD) for each parameter.

2.6. Analysis of dosage forms

For determination of 3-TC, abacavir sulphate and AZT in commercial dosage forms, the prepared sample solutions were analyzed using the developed method. 3-TC, abacavir sulphate and AZT content in commercial dosage form was determined using peak area of densitogram. Calibration curves were used for determination of the actual content of the three substances in the dosage forms so that the agreement of the results obtained with the label claim of the manufacturer can be evaluated.

3. Results and Discussions

The three drugs are some how polar in nature but 3-TC is relatively more polar than ABC and ABC in turn more polar than AZT. All the three drugs were not eluted from the site of application when single non polar solvents like n-hexane, toluene, dichloromethane and chloroform were used as a mobile phase. Hence, the use of additional relatively polar solvents like ethyl acetate, methanol, acetone and acetonitrile was important. A combination of two solvents, one non polar and the other relatively polar also was not enough to separate the two more polar drugs i.e. 3-TC and ABC. Thus it was necessary to use a third solvent to separate the two more polar drugs. Among the mobile phase combinations tried, the one containing methanol: acetonitrile: chloroform (4:3:8) gave well resolved compact bands for the drug mixture components. In the solvent system, all the three moved a suitable distance that was appropriate for scanning and hence good for quantitation purpose. The R_f values observed were 0.38, 58 and 0.71 for 3-TC, ABC and AZT respectively.

The UV-Visible spectrum of 3-TC, ABC and AZT were collected separately and in mixture in the ranges from 200 to 400 nm. The λ max for 3-TC, ABC and AZT were at 277 ± 2 nm, 288 ± 2 nm and 270 ± 2 nm respectively and the intersection point of their spectrums were also obtained. The peak area's for each drugs were evaluated at the three intersection wavelengths and λ max for each of the drugs. A good response was obtained at the intersection point between AZT and 3-TC i.e. 275± 2 nm. Quantitative determinations of 3-TC, ABC and AZT were performed by scanning the spots at 275 nm in absorbance/ reflectance mode (Fig. 2).

Test solution stability study was conducted using the dosage form solution over two day period of time. The average peak areas and relative standard deviation were determined and presented in Table 1. Statistical comparison between the data obtained from the sample spotted immediately after preparing the solution and the rest of the samples which were spotted some time after the solution was prepared and stored were performed. Since student t-test value of the average peak areas of 3-TC, ABC and AZT after 48 hours were (p= 0.05, t_{stat} = 4.33, 6.91 and 5.06 for 3-TC, ABC and AZT respectively) greater than that of 25 minute i.e. $t_{critical}$ (p= 0.05,

$t_{critical}$ = 2.36, 2.36 and 2.26 for 3-TC, ABC and AZT respectively), the average peak areas of 3-TC, ABC and AZT after 48 hours were significantly varied from the average peak areas obtained after 25 minutes of sample preparation. No other new peak appeared to indicate presence of degradation product in the study time. This suggests that the analysis should be carried out within 24 hours of sample preparation.

Table 1
Stability study of the dosage form solution (n=5).

Time of analysis	3-TC (300 ng/band)		ABC (600 ng/band)		AZT (600 ng/band)	
	Area	RSD (%)	Area	RSD(%)	Area	RSD (%)
25 minutes	5299.45	0.79	8356.05	0.67	6443.71	1.28
4 hours	5280.77	0.94	8339.15	0.85	6420.27	1.13
8 hours	5256.67	1.26	8320.07	0.89	6399.11	1.36
24 hours	5113.67	1.26	7987.49	1.48	6186.12	1.51
48 hours	5455.23	2.38	5728.46	2.42	3832.94	2.57

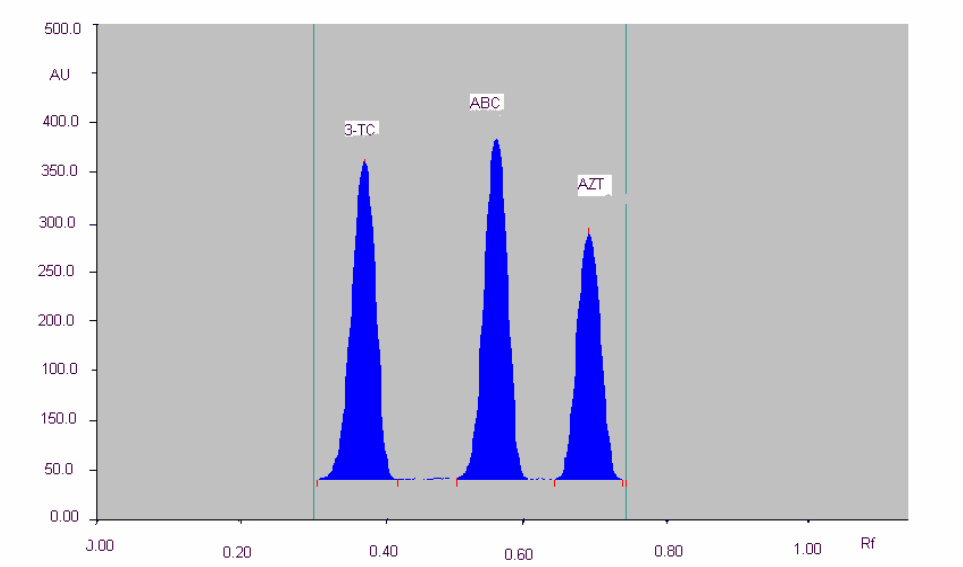

Fig. 2. Densitogram of lamivudine(3-TC), abacavir (ABC) and zidovudine (AZT)

The relationship between the concentration of each of 3-TC, ABC and AZT and peak area and peak height of the spots was investigated. The peak areas were found to have good relationship with the concentration than peak heights and thus peak area was used throughout this study. The linear relationship was tested and found to be less acceptable due to the minimal fitting of the residuals on the calibration line, indicating lower precise correlations and determinations (r = 0.9915, 0.9952 and 0.9915 and r^2 =0.9831, 0.9905 and 0.983 for 3-TC, ABC and AZT respectively). Thus, the linear model for fitting the data can be used but with less precise calculations. The characteristic parameters of the linear regression equation of the three drugs are shown in Table 2. The second order polynomial fit was found to be more suitable. Its

residuals plot showed a much better fitting than that of the linear model, indicating good correlation and determinations (r = 0.99995, 0.9998 and 0.9998 and r^2 = 0.9999, 0.9997 and 0.9996 for 3-TC, ABC and AZT, respectively). The calibration graphs were constructed in the range of 200–1450 ng/spot for 3-TC, ABC and AZT. Characteristic parameters of the second order polynomial regression equation of the three drugs are shown in Table 3.

Table 2
Characteristic parameters for the linear regression equations obtained from the HPTLC-densitometric method for the studied drug (*n=5*)

Parameters	3-TC	ABC	AZT
Linearity range (ng/band)	200-1450	200-1450	200-1450
Determination coefficients (r^2)	0.9831	0.9905	0.983
Correlation coefficients (r)	0.9915	0.9952	0.9915
Coefficient of x ± S.D	8.182 ± 0.5358	8.6581 ± 0.2164	7.8405 ± 0.2600
Intercept ± S.D.	3127.7 ± 117.8	2941.2 ± 151.08	1496.8 ± 141.53
Linear regression equation	8.182x + 3127.7	8.6581x + 2941.2	8.18405x + 1496.8

Table 3
Characteristic parameters for the second order polynomial regression equations obtained from the HPTLC-densitometric method for the studied drug (*n=5*)

Parameters	3-TC	ABC	AZT
Linearity range (ng/band)	200-1450	200-1450	200-1450
Determination coefficients (r^2)	0.9999	0.9997	0.9996
Correlation coefficients (r)	0.9999	0.9998	0.9998
Coefficient of x^2 ± S.D	-0.0029±0.00013	-0.0023±0.00014	-0.0028±0.00014
Coefficient of x ± S.D	13.007 ± 0.38	12.427 ± 0.3283	12.453 ± 0.2033
Intercept ± S.D.	1670.3 ± 42.91	1803.1 ± 5214	103.31 ± 43.38

Repeatability of sample application and measurement of peak area were expressed in terms of relative standard deviation and were found to be very low 0.45, 0.21 and 0.33 and 0.46, 0.19 and 0.24 for 3-TC, ABC and AZT, respectively (Table 4).

This indicted reproducible performance of the instrument. Variation between analysts was also studied by making the sample to be analyzed by three analysts. The sample was analyzed by the three analysts within a day and the results revealed that there is good intermediate precision between analysts (Table 5) with relative standard deviation of 1.06, 0.87 and 1.5 for 3-TC, ABC and AZT respectively. Repeatability and intermediate precision of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. The results showed that intra- and inter-day variation of the results at three different concentration levels of 300, 600, 12000 ng/spot for 3-TC, ABC and AZT was with in the acceptable range. RSD for both the inter-day and intra-day precision of the method was found to be < 2 % for both drugs as indicated in Table 6.

Table 4
Results for system precision studies of the developed method (n=6).

	Repeatability of sample application			**Repeatability of measurement of peak area**		
No	**Area of 3-TC at 300(ng/band)**	**Area of ABC at 600(ng/band)**	**Area of AZT at600 (ng/band)**	**Area of 3-TC at 300 (ng/band)**	**Area f ABC at 600 (ng/band)**	**Area of AZT at 600(ng/band)**
1	5287.98	8453.90	6487.85	5297.06	8481.78	6484.41
2	5296.43	8467.07	6493.68	5288.22	8476.64	6491.52
3	5267.12	8425.35	6473.35	273.93	8465.23	6476.21
4	5259.54	8470.32	6469.83	5253.66	8454.29	6468.32
5	5245.23	8445.55	6453.39	5247.30	8446.38	6472.42
6	5234.89	8434.88	6436.87	236.45	8441.58	6445.88
Mean	5265.19	8449.51	6469.16	5266.1	8460.98	6473.13
SD	23.86	17.74	21.26	24.1	16.31	15.76
RSD(%)	0.45	0.21	0.33	0.46	0.19	0.24

The limit of detection of 3-TC, ABC and AZT were found to be 47.51, 57.58 and 59.57 ng/band respectively while the limit of quantification for TC, ABC and AZT was found to be 143.97, 174.5 and 180.51 respectively. Peak purity test of 3-TC, ABC and AZT spots were assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot of the dosage form and standards [25]. The results indicated that the peaks are so pure and the respective correlation coefficients were (0.9998, 0.9999 and 0.9998 at peak start and apex, 0.9996, 0.9994 and 0.9995 at peak apex and peak end for 3-TC, ABC and AZT, respectively and this indicate that there is good correlation between standard and sample spectra (Fig. 3).

Table 5
Results for the study of variation between analysts (n=5).

Analyst	**3-TC (300.00ng/band)**		**ABC (600.00ng/band)**		**AZT (600.00ng/band)**	
	Mean peak area (AU)	RSD (%)	Mean peak area (AU)	RSD (%)	Mean peak area (AU)	RSD(%)
1	5625.34	1.51	8425.73	0.87	6505.37	1.47
2	5184.07	1.89	8545.50	1.35	6442.16	1.56
3	5290.74	1.925	8368.78	1.28	6359.25	1.07
RSD*		1.06		0.87		1.50

RSD* is the relative standard deviation between analysts for each substances.

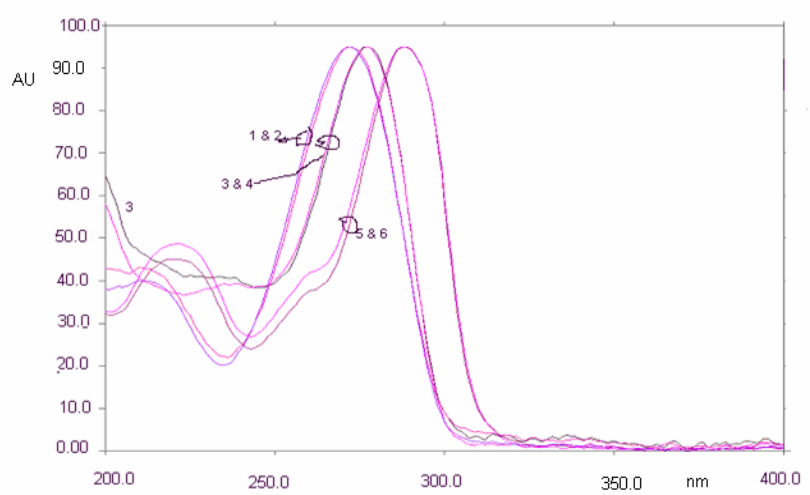

Fig. 3. UV spectra comparison of the spots of the standards (1,3&5) and dosage form (2,4&6) for zidovudine, lamivudine and abacavir respectively.

Table 6
Method precision studies of the developed method.

Substance	Amount (ng/band)	Intra-day precision (n=6)		Inter-day precision (n=3)	
		Mean peak area (AU)	RSD (%)	Mean peak area (AU)	RSD (%)
3-TC	300.00	5284.76	1.6783	5265.34	1.51
	600.00	8457.39	1.4113	8453.20	1.01
	1200.00	13017.78	1.9102	13000.30	0.79
ABC	300.00	5275.98	1.1990	5273.50	1.24
	600.00	8456.58	1.2287	8425.73	0.87
	1200.00	13122.71	1.4683	13120.83	1.17
AZT	300.00	3540.68	1.3808	3540.90	1.97
	600.00	6547.82	1.3462	6505.37	1.47
	1200.00	10970.67	1.3998	10972.75	1.10

Accuracy of the method was evaluated using percentage recovery. Percentage recovery (Table 7) at each level was calculated and found to be acceptable. Values obtained for recoveries ranged from 99.13 to 99.49 for 3-TC, 98.93 to 99.39 for ABC and 98.69 to 99.43 for AZT. The results indicated absence of interferences from the common pharmaceutical excipients used in the manufacture of the selected formulations. Recovery experiments from tablets also showed the reliability and suitability of the method and such acceptable recovery values with low relative standard deviation (1.3%, for all drugs) indicate that, the method provides sufficient accuracy.

The effect of volume and composition of the mobile phase, time from spotting to development and from development to scanning on R_f values and peak area of 3-TC, ABC and AZT were used to study robustness of the proposed method. The relative standard deviation of peak areas and R_f values were calculated for each parameter and the average RSD was found to be less than 2% (Table 8). This indicated that the proposed method is robust. From the studied parameters, a small but deliberate variation ($\pm$10%) in total volume of the mobile phase resulted in larger RSD than the others and this revealed that volume of the mobile phase is a very sensitive parameter during the optimization process. The effects of a 10% variation of volume of the mobile phase caused larger RSD in R_f values (2.73) than in peak area (1.74).

The proposed method was applied to the determination of 3-TC, ABC and AZT in commercial tablets of Trizivir. Five replicate determinations were made and good results were obtained for all drugs and were in agreement with the label claims.

Table 7
Data for recovery study by standard addition technique for the developed method using the dosage form (n=5).

Samples	**Theoretical amount (ng/band)**	**Amount of std added to the analyte (ng)**	**% of std added to the analyte**	**Average response (area)**	**% recovery**	**RSD (%)**
3-TC	300.00	0	0	5277.08	99.36	0.5786
	300.00	240.00	80	7802.24	99.40	0.8249
	300.00	300.00	100	8387.64	99.49	1.0170
	300.00	360.00	120	8913.39	99.13	1.3040
ABC	600.00	0	0	8341.40	98.93	0.7664
	600.00	480.00	80	12439.92	99.19	0.8267
	600.00	600.00	100	13303.51	99.25	0.9479
	600.00	720.00	120	14112.99	99.39	1.0894
AZT	600.00	0	0	6481.90	98.69	0.8386
	600.00	480.00	80	10195.65	99.11	0.9862
	600.00	600.00	100	10937.67	99.29	1.1490
	600.00	720.00	120	11597.79	99.43	1.2282

Table 8
Robustness study of the method (n=5).

Parameters	**R_f values RSD(%)**			**Average area** RSD(%)		
	3-TC	ABC	AZT	3-TC 300 (ng/band)	ABC 600 (ng/band)	AZT 600 (ng/band)
Composition of mobile phase	2.73	1.94	1.07	1.74	1.06	1.22
Volume of mobile phase	1.79	2.44	1.99	1.65	1.11	1.65
Time from development to scanning(5 to 60 minutes)	2.13	1.44	1.01	1.30	0.93	1.17
Time from spotting to development (5 to 60 minutes)	1.39	0.94	0.63	1.55	0.09	1.28

As indicated in Table 9, the drug content was found to be 99.20 % ± 0.98 (RSD = 0.98), 99.04% ± 1.44 (RSD = 1.45) and 98.64% ± 1.56 (RSD = 1.58) for 3-TC, ABC and AZT, respectively. The low RSD indicated the suitability of this method for routine analysis of the three drugs in pharmaceutical dosage forms.

Table 9
Assay results of the commercial dosage forms (n=5).

Parameters	3-TC	ABC	AZT
Label claim (mg/tablet)	150.00	300.00	300.00
Drug content (%) ± S.D	99.20 ± 0.98	99.04 ± 1.44	98.64 ± 1.56
RSD (%)	0.98	1.45	1.58

4. Conclusion

The developed HPTLC method provides simple, precise and accurate analysis method for the simultaneous determination of 3-TC, ABC and AZT in the combined dosage form (tablet). Statistical analysis proved that the method is repeatable, accurate, robust and specific for the analysis of lamivudine, abacavir and zidovudine in pharmaceutical formulations (tablet).

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