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A New RP- HPLC Method for the Simultaneous Estimation of Abacavir, Lamivudine and Zidovudine in Tablet Dosage Forms.

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ABSTRACT

An accurate, precise and reproducible high performance liquid chromatographic method was developed for quantitative estimation of abacavir, lamivudine and zidovudine simultaneously in tablet dosage forms. Separation of the drugs was achieved within 15.0 min on a Hichrom RP-select B column (250 x 4.6 mm; 5µ) by gradient elution using mixtures of 0.02M ammonium acetate and methanol as the mobile phase. The analytes in the eluate were monitored at 250 nm. The retention times obtained for abacavir, lamivudine and zidovudine were 12.172, 1.884 and 4.378 min respectively. The calibration curves were linear over the range of 25-200 µg/mL for abacavir, 12.5-100 µg/mL for lamivudine and 25-200 µg/mL for zidovudine. The performance of the method was validated according to ICH guidelines. The method was found to be suitable for accurate determination of these drugs in tablet dosage forms without any interference from the excipients or endogenous substances.

Key words: Abacavir, Lamivudine, Zidovudine, Determination, HPLC, Gradient elution.

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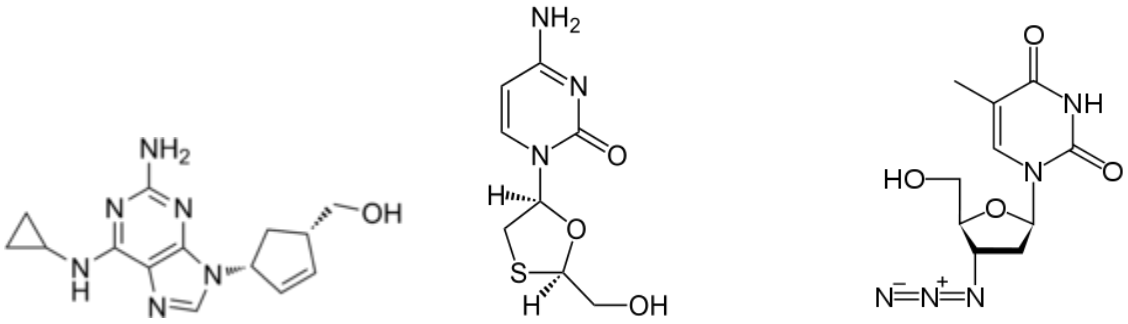
INTRODUCTION

The synthetic antiretroviral drug analogues abacavir[1], lamivudine[2] and zidovudine[3] (Figure 1) form one of the fixed dosage combinations used in the effective management of HIV. Their active triphosphate metabolites act against HIV by inhibiting the activity of HIV-1 reverse transcriptase both by competing with its natural substrate dGTP & by their incorporation into the viral DNA.

A literature survey revealed that many analytical methods have been reported for the determination of abacavir, lamivudine and zidovudine in dosage forms and in biological samples using, spectrophotometry[4,5] and liquid chromatography[6-16] either singly or in combination**.**

We have now developed a new RP-HPLC method for simultaneous estimation of abacavir, lamivudine and zidovudine using gradient elution technique in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the ICH [17] guidelines.



(a) **(b)** **(c)**

Figure 1: **Chemical structures of abacavir (a), lamivudine (b) and zidovudine (c).**

MATERIALS AND METHODS

Chemicals, solvents and drugs

Ammonium acetate (extra pure) was purchased from Qualigens Chemicals Limited. HPLC grade methanol was purchased from Merck Limited. HPLC grade water was prepared by using Millipore Milli-Q system. Working standard samples of abacavir, lamivudine and zidovudine were obtained from Hetero Labs Ltd. (Hyderabad, India). The commercial tablets formulation Trizivir was purchased from the local market.

Equipment and chromatographic conditions

The chromatographic system employed for this study consisted of Waters Alliance liquid chromatograph (model 2695) fitted with a Hichrom RP-Select B column (250 x 4.6 mm; 5µ) and a diode array detector (model 2996) using Empower2 software for data handling. All chromatographic runs were carried out in gradient mode with a flow rate of 0.8 mL/min. The

mobile phase used for separation of the three drugs consisted of mixtures of 0.02M ammonium acetate (mobile phase-A) and methanol (mobile phase-B). The 0.02M ammonium acetate buffer was prepared by dissolving 1.54g of ammonium acetate in 1000mL of water, followed by filtration through a 0.45µ filter and sonication. The injection volume was 10µL and the column temperature was maintained at 40°C. The detector wavelength was set at 250 nm for monitoring the analytes.

Preparation of the diluent

A 50:50 v/v mixture of water and methanol was used as the diluent for preparing drug solutions.

Preparation of working standard solution of abacavir, lamivudine and zidovudine

100 mg of abacavir, 50 mg of lamivudine and 100 mg of zidovudine were accurately weighed and transferred into a 100 mL volumetric flask. 50 mL of the diluent was added to it and the drugs were dissolved completely by sonication. The volume was made up with a further quantity of the diluent and mixed well. This was used as standard stock solution. 5.0 mL of this solution was transferred into a 50 mL volumetric flask, diluted to volume with the diluent to get concentrations of 100 µg/mL, 50 µg/mL and 100µg/mL of abacavir, lamivudine and zidovudine respectively. This was used as the working standard solution.

Calibration curve

Mixed standard solutions of abacavir, lamivudine and zidovudine were prepared at different concentration levels including working concentration mentioned in experimental condition. Ten microlitres of each concentration was injected into the HPLC system. The responses were read at 250nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.

Estimation of the drugs from tablet dosage forms

Ten Trizivir tablets were weighed and ground to a fine powder. From this, an amount equivalent to about 100 mg of abacavir was transferred into a 100 mL volumetric flask and to it 75 mL of the diluent was added and sonicated for 20 min. The diluent was further added to make up the volume and mixed. A portion of the above solution was filtered through 0.22 µm membrane filter (discarding the first few mL of the filtrate). 5mL of this filtrate was transferred into a 50 mL volumetric flask containing 30 mL of the diluent. The volume was made up to 50 mL with the diluent and mixed well. The above solution was then chromatographed for six times. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by using the regression equations of the linearity plots.

RESULTS AND DISCUSSION

Using mobile phase-A and B in gradient mode as shown in the Table 1, base line separation for the peaks of lamivudine, zidovudine and abacavir was achieved. Under these conditions, the retention times for lamivudine, zidovudine, and abacavir were found to be 1.884, 4.378, and12.172 min respectively. The proposed method was also applicable to tablet formulations. Stationary Phase: Hichrom RP-Select B (250 x 4.6 mm, 5µ) Mobile Phase-A: 0.02M Ammonium Acetate, Mobile Phase-B: 100% Methanol

Table 1: Proposed HPLC method

Gradient Program			
S.No.	**Time (min)**	**Mobile Phase-A**	**Mobile Phase-B**
1	1	85	15
2	10	70	30
3	15	30	70
4	22	30	70
5	23	85	15
6	30	85	15

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of suspected interferences such as excipients and degradation products. Figure. 3 shows chromatographic base-line separation of abacavir, lamivudine and zidovudine. Figure 4 demonstrates that no interference was found at the retention times of abacavir, lamivudine and zidovudine in their dosage forms due to excipients.

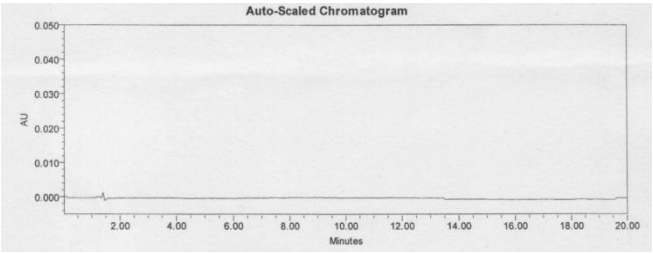


Figure 2: Chromatogram obtained from the blank

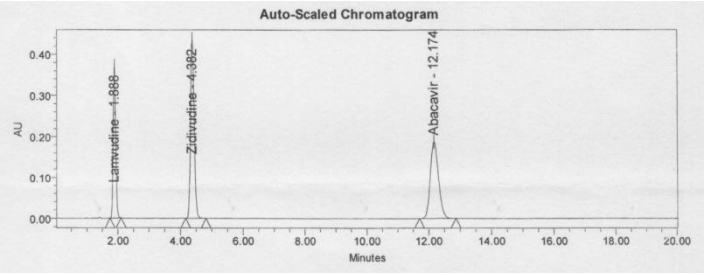


Figure 3: chromatogram obtained from analysis of abacavir, lamivudine and zidovudine from working standard solution.

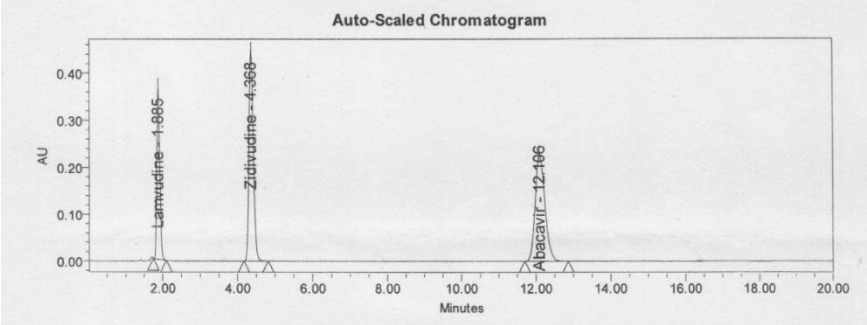


Figure 4: Representative chromatogram obtained from analysis of abacavir, lamivudine and zidovudine from formulation sample solution..

Linearity

The regressions of the plots were computed by least square regression method and were shown in the Figures 5, 6 and 7. The calibration curves (n=3) constructed for each drug were linear over the concentration range of 25-250 µg/mL for abacavir, 12.5-125 µg/mL for lamivudine and 25-250 µg/mL for zidovudine. The correlation coefficients were greater than 0.999 and the %RSD for each concentration studied was less than 2%.

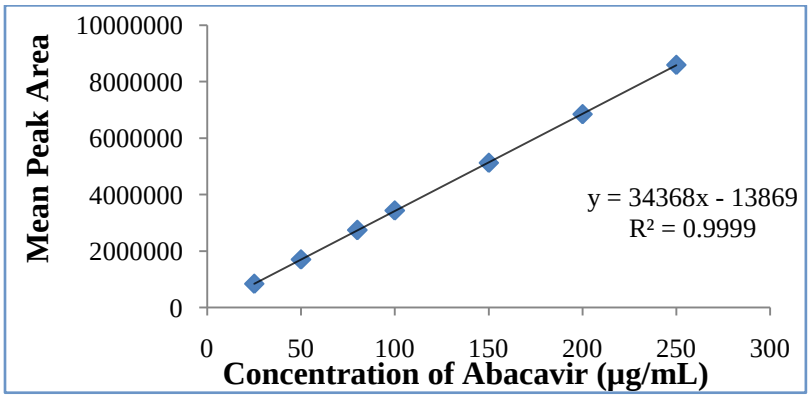


Figure 5: Linearity plot for abacavir

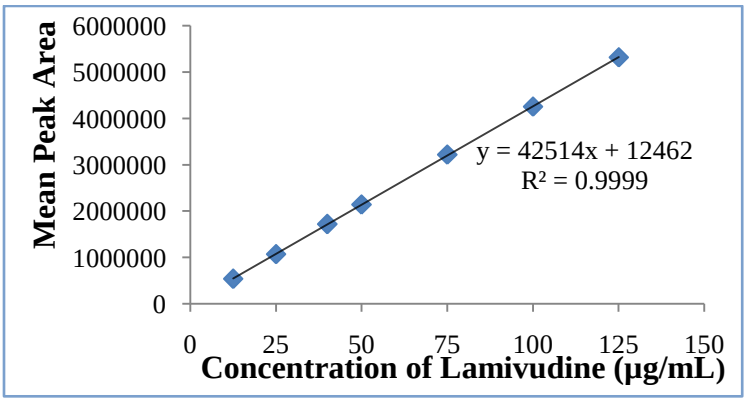


Figure 6: Lineaity plot for lamivudine

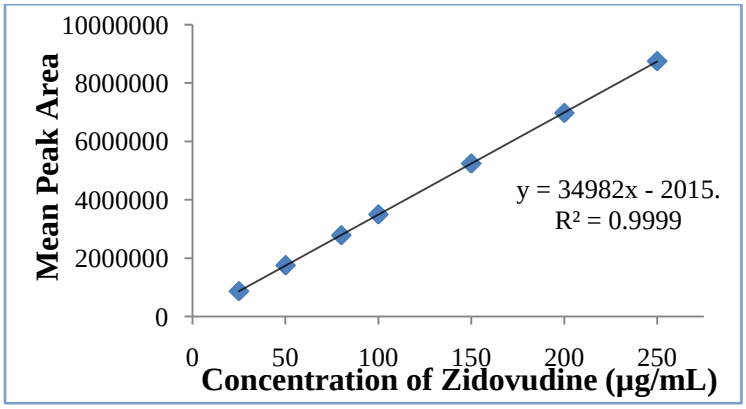


Figure 7: Linearity plot for zidovudine

Table 2: Accuracy data of the proposed method

Analyte	Amount of the analyte taken(µg/mL)	Mean recovery (µg/mL) ± SD	% Mean recovery ± SD
Abacavir	80	80.16 ± 0.110	100.20 ± 0.137
	100	100.21 ± 0.028	100.21 ± 0.028
	120	120.19 ± 0.170	100.16 ± 0.141
Lamivudine	40	40.02 ± 0.100	100.05 ± 0.250
	50	50.02 ± 0.188	100.04 ± 0.376
	60	60.05 ± 0.092	100.08 ± 0.153
Zidovudine	80	80.16 ± 0.072	100.20 ± 0.090
	100	100.05 ± 0.098	100.05 ± 0.098
	120	120.15 ± 0.036	100.12 ±0.030

Table 3: Precision data for the proposed method

		Intra-day precision		Inter-day precision	
Analyte	**Concentration taken (µg/mL)**	**Measured concentration (µg/mL) ± SD**	**%RSD**	**Measured concentration (µg/mL) ± SD**	**%RSD**
Abacavir	50	49.88 ± 0.218	0.44	49.64 ± 0.179	0.36
	100	100.63 ± 0.220	0.22	100.53 ± 0.101	0.10
	150	149.95 ± 0.290	0.19	149.79 ± 0.132	0.10
Lamivudine	25	24.86 ± 0.108	0.44	24.82 ± 0.033	0.13
	50	50.11 ± 0.115	0.23	50.00 ± 0.071	0.14
	75	75.24 ± 0.195	0.26	75.39 ± 0.087	0.12
Zidovudine	50	50.05 ± 0.120	0.24	50.11 ± 0.016	0.03
	100	100.12 ± 0.270	0.27	100.39 ± 0.243	0.24
	150	150.08 ± 0.151	0.10	150.19 ± 0.144	0.10

Table 4: System suitability parameters of the proposed method

Parameter	Abacavir	Lamivudine	Zidovudine
Retention time (min)	12.172	1.884	4.378
Tailing factor	1.1	1.1	1.1
Theoretical plates	10351	2491	6910
HETP	0.0024	0.0100	0.0036

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in table 2. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Three replicate injections of sample solutions were made and the percentage RSD was calculated and represented in Table 3. From the data obtained the developed RP-HPLC method was found to be precise.

System Suitability Parameters

System suitability parameters were studied with six replicates of standard sample solutions and the parameters are presented in Table 4.

Limit of Detection and Limit of Quantitation

Limit of detection (LOD) is the smallest level of analyte that gives a measurable response. Limit of quantitation (LOQ) is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. LOD and LOQ are based on S/N ratio (signal/noise) for HPLC methods. LOD is the concentration at which analyte response is 3.3 times greater than noise. LOQ is the concentration at which analyte response is 10 times greater than noise. Six replicates of the analyte were analyzed and quantified. The results are shown in Table 5.

Table 5: Limit of detection and quantitaion

Analyte	LOD (µg/mL)	LOQ (µg/mL)
Abacavir	1.210	3.666
Lamivudine	0.861	2.610
Zidovudine	1.057	3.202

Method Suitability

The commercial tablet formulation, Trizivir was analyzed by the proposed method and the results are shown in table 6. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of drugs in pharmaceutical dosage forms.

Table 6: Recovery of abacavir, lamivudine and zidovudine

Drug	Labeled amount (mg)	Amount recovered (n=6)	% Recovery
Abacavir	300	299.35	99.78
Lamivudine	150	150.25	100.16
Zidovudine	300	298.75	99.58

CONCLUSION

The proposed RP –HPLC method is sensitive, precise and accurate and can be used for the

routine quality control analysis for the simultaneous determination of the abacavir, lamivudine and zidovudine in their tablet dosage forms.

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