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A NEW, SIMPLE, SENSITIVE, ACCURATE & RAPID ANALYTICAL METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, ABACAVIR & ZIDOVUDINE IN TABLET DOSAGE FORM BY USING UPLC

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Keywords:

Abacavir, Lamivudine, Zidovudine, LOD, LOQ, UPLC

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ABSTRACT: The present work was undertaken to develop and validate a rapid and consistent UPLC method in which the peaks will appear in a short period as per ICH Guidelines. The UPLC separation was achieved on a Symmetry C_{18} (2.1 × 100mm, 1.7µm, Make: BEH) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (60%) [pH 3.0] & Methanol (40%) [UPLC Grade] The flow rate was monitored at 0.25 ml per min. The wavelength was selected for the detection was 280 nm. The run time was 3 min. The retention time found for the drugs Lamivudine, Abacavir, and Zidovudine was 1.019 min, 1.271 min & 1.617 min respectively. The % recovery was found to be 98.0%- 99.0% for the drug Abacavir. The % recovery was found to be 98.0% - 99.6% for the drug Lamivudine. The % recovery was found to be 98.2% - 98.6% for the drug Zidovudine. The linearity was established in the range of 20 to 60 ppm for the drug Abacavir & 10 to 30 ppm for the drug Lamivudine & 20 to 60 ppm for the drug Zidovudine. The LOD for the drugs Abacavir, Lamivudine, and Zidovudine were found to be 0.002 µg/ml, 0.003 µg/ml, & 0.005 µg/ml, respectively. The LOQ for the drugs Abacavir, Lamivudine, and Zidovudine were found to be 0.008 µg/ml, 0.01 µg/ml & 0.02 µg/ml respectively. Overall the proposed method was found to be suitable, sensitive, reproducible, specific and accurate for the quantitative determination of the drug in tablet dosage form.

INTRODUCTION: Abacavir, Lamivudine, and Zidovudine are synthetic nucleoside analogs showing a potent and synergistic effect on inhibition of the human immunodeficiency virus (HIV-1), the causative agent of acquired immunodeficiency syndrome [1] (AIDS).

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HIV encodes at least three enzymes: protease, reverse transcriptase, and endonuclease. The Abacavir, Lamivudine, and Zidovudine belong to the class of nucleoside reverse transcriptase inhibitors (NRTI). New therapeutic strategy of AIDS treatment requires the combination of these antiretroviral (ARV) drugs. The introduction of highly effective combination regimens of ARV drugs has led to substantial improvements in morbidity and mortality. The formulations contain three nucleoside analogs (Abacavir sulfate, Lamivudine, and Zidovudine) [2] and are intended for patients whose regimen would otherwise

include these three components. Abacavir is converted by cellular enzymes to the active metabolite, carbovirtriphosphate (CBV-TP), an analog of deoxyguanosine-5'-triphosphate (dGTP). Intracellularly, Lamivudine is phosphorylated to its active 5'- triphosphate metabolite, Lamivudine triphosphate (3TC-TP). Intracellularly, Zidovudine is phosphorylated to its active 5'-triphosphate metabolite, Zidovudine triphosphate (ZDV-TP). The chemical name of Abacavir sulfate is (1 S, cis)-4-[2-amino-6- (cyclopropyl amino)-9 H -purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1).

It has a molecular formula of $(C_{14}H_{18}N_6O)_2 \bullet H_2SO_4$ and a molecular weight of 670.76 Daltons. Lamivudine is (2R, cis)-4- amino-1-(2-hydroxy-methyl-1, 3-oxathiolan-5-yl)- (1H)- pyrimidin-2-one. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3 Daltons. Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in the water at 20 °C. Zidovudine is 3 ′ -azido-3′ -deoxythymidine [2]. It has a molecular formula of $C_{10}H_{13}N_5O_4$ and a molecular weight of 267.24 Daltons. It is a white to beige, crystalline solid with a solubility of 20.1 mg/mL in the water at 25 °C.

Numerous analytical methods employed like spectrophotometry [3] and liquid chromatography [4-14] have been reported of individual or multi-component combinations assay of NRTI in biological fluids and pharmaceutical dosage forms. The reported LC the eluent used for RP-HPLC and the UV detection wavelength. The development and validation of a simple, rapid, accurate and precise combined assay for Abacavir, Lamivudine, and Zidovudine in tablet formulations are now reported in this paper using UPLC with UV detection at 280 nm methods differ concerning the extraction procedure. Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about 9-fold decreases in analysis time as compared to the conventional HPLC system using 5 µm particle size analytical columns, and about 3-fold decrease in analysis time in comparison with 3 µm particle size analytical columns without compromise on overall separation.

The chemical structures for the drug were represented in **Fig. 1, 2** and **3**.

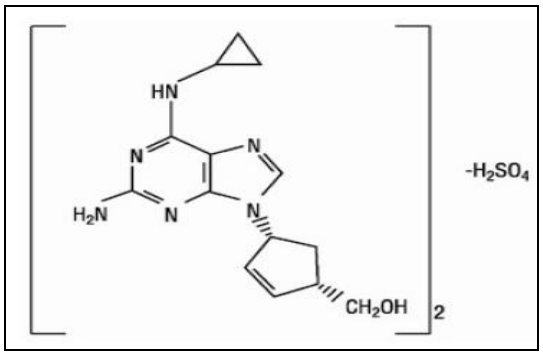

FIG. 1: CHEMICAL STRUCTURE OF ABACAVIR SULFATE

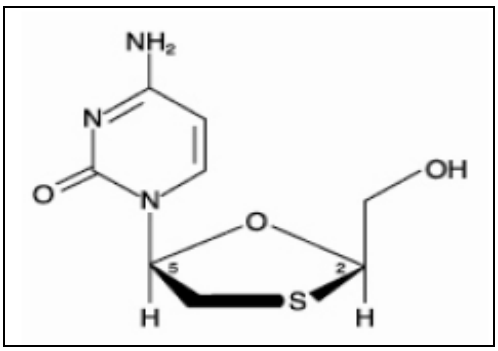

FIG. 2: CHEMICAL STRUCTURE OF LAMIVUDINE

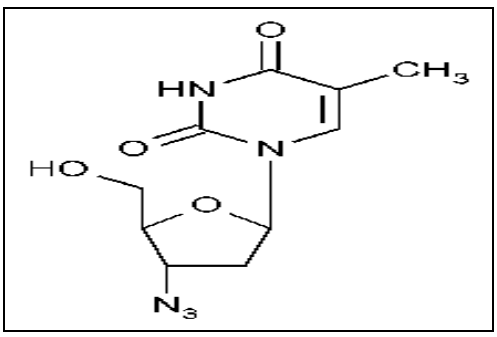

FIG. 3: CHEMICAL STRUCTURE OF ZIDOVUDINE

MATERIALS & METHOD:

Chemicals and Reagents Used: The following chemicals were procured for the process: Water [UPLC Grade], Methanol [UPLC Grade], Methanol [UPLC Grade], Abacavir, Lamivudine and Zidovudine [Working standards], Orthophosphoric Acid & Potassium Dihydrogen Phosphate all the chemicals were procured from STANDARD SOLUTIONS, and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:

Equipment: Ultra performance liquid chromato-graphy equipped with Auto Sampler and DAD or UV detector.

UV/VIS Spectrophotometer: LAB INDIA UV 3000^{+}

pH Meter: Adwa – AD 1020

Weighing Machine: Afcoset ER-200A

Temperature: Ambient

Column: Symmetry C_{18} (2.1 x 100mm, 1.7µm, Make: BEH) or equivalent

Phosphate Buffer: 7.0 grams of Potassium Dihydrogen Phosphate in 1000 ml Water [HPLC Grade] pH adjusted with Orthophosphoric Acid.

pH: 3.0

Mobile Phase: Phosphate Buffer: Methanol (60: 40v/v)

Flow Rate: 0.25 ml per min

Wavelength: 280 nm

Injection Volume: 2 µl

Run Time: 3 min.

Preparation of Phosphate Buffer: The buffer solution was prepared by dissolving accurately weighed 7.0 grams of Potassium Dihydrogen Phosphate and transferred into a clean and dry 1000 ml volumetric flask, dissolved and diluted with 1000 ml water [UPLC Grade]. The final pH of the buffer was adjusted to 3.0 by using Ortho Phosphoric Acid.

Preparation of Mobile Phase: The Mobile Phase was prepared by mixing 600 ml (60%) of the above buffer and 400 ml of Methanol [UPLC Grade] (40%) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through a 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as Diluent.

Preparation of the Abacavir, Lamivudine, and Zidovudine Standard & Sample Solution:

Preparation of Stock Solution: The stock solution was prepared by weighing accurately 5 mg Abacavir, 10 mg Lamivudine, and 10 mg Zidovudine and transferred into a clean and dry 10 ml volumetric flask. About 7 ml of diluent was added and sonicated. The volume was made up to the mark with the same diluent. From the above-prepared Stock solution pipette out 0.4 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark to get final concentration.

Preparation of Sample Solution: The sample solution was prepared by weighing equivalently 1635.8 mg of Abacavir, Lamivudine, and Zidovudine and transferred into a 100 ml clean and dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve it completely, and the volume made up to the mark with the same solvent. From above-prepared stock solution pipette out 0.13 ml of the solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10 ml to get final concentration. The standard and sample solutions were injected five times, and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability: The Tailing factor for the peaks due to Abacavir, Lamivudine, and Zidovudine in Standard solution should not be more than 1.5. The Theoretical plates for the Abacavir, Lamivudine, and Zidovudine peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Abacavir, Lamivudine and Zidovudine standard. The parameters of system suitability were checked.

Assay calculation for Abacavir, Lamivudine, and Zidovudine:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.Wt.}}{\text{Label Claim}} \times 100$$

Where:
AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of the test taken in mg.
DS = Dilution of standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

System Suitability Results for Abacavir: The Tailing factor obtained from the standard injection was 1.4.

The Theoretical Plates obtained from the standard injection was 3828.5.

Assay Result for Abacavir:

$$\frac{1395596}{1364065}\times\frac{10}{10}\times\frac{0.4}{10}\times\frac{7}{10}\times\frac{100}{1635.8}\times\frac{10}{0.13}\times\frac{99.8}{100}\times\frac{1635.8}{300}\times 100 = 100.8\%$$

System Suitability Results for Lamivudine: The Tailing factor obtained from the standard injection was 1.4.

The Theoretical Plates obtained from the standard injection was 2842.6

Assay Result for Lamivudine:

$$\frac{1203186}{1209273}\times\frac{5}{10}\times\frac{0.4}{10}\times\frac{100}{1635.8}\times\frac{10}{0.13}\times\frac{99.9}{100}\times\frac{1635.8}{150}\times 100 = 98.1\%$$

System Suitability Results for Zidovudine: The Tailing factor obtained from the standard injection was 1.3.

The Theoretical Plates obtained from the standard injection was 6452.0.

Assay Result for Zidovudine:

$$\frac{1262423}{1258415}\times\frac{10}{10}\times\frac{0.4}{10}\times\frac{100}{1635.8}\times\frac{10}{0.13}\times\frac{99.6}{100}\times\frac{1635.8}{300}\times 100 = 98.6\%$$

Validation Development: [15-16]

Precision: It is a measure of the degree of repeatability of an analytical method under normal operation, and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in **Fig. 7 Table 1, 2** and **3**.

TABLE 1: PRECISION RESULT FOR THE DRUG ABACAVIR

Injection	Area
Injection-1	1361495
Injection-2	1359608
Injection-3	1362229
Injection-4	1364566
Injection-5	1360414
Average	1361662
Standard Deviation	1907.8
%RSD	0.14

TABLE 2: PRECISION RESULT FOR THE DRUG LAMIVUDINE

Injection	Area
Injection-1	1203297
Injection-2	1205940
Injection-3	1200143
Injection-4	1202351
Injection-5	1208316
Average	1204009
Standard Deviation	3181.2
%RSD	0.26

TABLE 3: PRECISION RESULT FOR THE DRUG ZIDOVUDINE

Injection	Area
Injection-1	1250339
Injection-2	1251830
Injection-3	1253995
Injection-4	1254313
Injection-5	1252122
Average	1252520
Standard Deviation	1641.7
%RSD	0.13

Intermediate:

Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on a different day by using different make column of same dimensions. The standard solution was injected five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in **Fig. 8, Table 4, 5** and **6**.

TABLE 4: RUGGEDNESS RESULT FOR THE DRUG ABACAVIR

Injection	Area
Injection-1	1391554
Injection-2	1393447
Injection-3	1386758
Injection-4	1391275
Injection-5	1389894
Average	1390586
Standard Deviation	2486.4
%RSD	0.17

TABLE 5: RUGGEDNESS RESULT FOR THE DRUG LAMIVUDINE

Injection	Area
Injection-1	1197958
Injection-2	1202356
Injection-3	1203211
Injection-4	1200063
Injection-5	1202962
Average	1201310
Standard Deviation	2248.7
%RSD	0.18

TABLE 5: RUGGEDNESS RESULT FOR THE DRUG ZIDOVUDINE

Injection	Area
Injection-1	1258204
Injection-2	1260921
Injection-3	1264320
Injection-4	1257560
Injection-5	1260545
Average	1260310
Standard Deviation	2669.5
%RSD	0.21

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a true conventional value or an accepted reference value and value found. The standard solution with Accuracy -80%, Accuracy -100% and Accuracy -120% was injected into the chromatographic system and calculated the amount found and amount added for Abacavir, Lamivudine, and Zidovudine and further calculated the individual recovery and mean recovery values. The chromatograms were represented in **Fig. 9, 10** and **11, Table 7, 8** and **9**.

TABLE 7: ACCURACY RESULT FOR THE DRUG ABACAVIR

% Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	7.90	7.80	99.0%	
100%	9.90	9.76	98.6%	98.5%
120%	11.9	11.6	98.0%	

TABLE 8: ACCURACY RESULT FOR THE DRUG LAMIVUDINE

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	188409	4.0	3.98	99.6%	
100%	2355870	5.0	4.97	99.4%	99.0%
120%	2785337	6.0	5.88	98.0%	

TABLE 9: ACCURACY RESULT FOR THE DRUG ZIDOVUDINE

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	1970294	8.0	7.89	98.6%	
100%	2458924	10.0	9.84	98.4%	98.4%
120%	2920382	11.9	11.6	98.2%	

Linearity: The method can elicit test result that is directly proportional to analyte concentration within a given range. It is generally reported as the variance of slope or regression line. It is determined by a series of three to six injections of five or more standards. Different levels of solution were prepared and injected to the chromatographic system, and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in **Fig. 12, 13** and **14**, **Table 10, 11** and **12**.

TABLE 10: LINEARITY CURVE FOR THE DRUG ABACAVIR

S. no.	Linearity Level	Concentration	Area
1	I	20ppm	510069
2	II	30ppm	930531
3	III	40ppm	1358156
4	IV	50ppm	1795688
5	V	60ppm	2228941
Correlation Coefficient			0.999

TABLE 11: LINEARITY CURVE FOR THE DRUG LAMIVUDINE

S. no.	Linearity Level	Concentration	Area
1	I	10ppm	454299
2	II	25ppm	824877
3	III	20ppm	1199471
4	IV	25ppm	1585444
5	V	30ppm	1961446
Correlation Coefficient			0.999

TABLE 12: LINEARITY CURVE FOR THE DRUG ZIDOVUDINE

S. no.	Linearity Level	Concentration	Area
1	I	20ppm	469196
2	II	30ppm	856104
3	III	40ppm	1248535
4	IV	50ppm	1651071
5	V	600ppm	2046212
Correlation Coefficient			0.999

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Detection for the drugs Abacavir, Lamivudine, and Zidovudine: The lowest concentration of the sample was prepared concerning the baseline noise and measured the signal to noise ratio. Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics) The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

$$\text{Limit of detection (LOD)} = \sigma \times 3.3 / S$$

Where:
S – Slope of the calibration curve
σ – Residual standard deviation

Calculation of S/N Ratio for Abacavir: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOD solution (0.26% of target assay concentration): 156 µV

$$S/N = 156/52 = 3.0$$

Calculation of S/N Ratio for Lamivudine: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOD solution (0.62% of target assay concentration): 154 µV

$$S/N = 154/52 = 2.96$$

Calculation of S/N Ratio for Zidovudine: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOD solution (0.62% of target assay concentration): 161 µV

$$S/N = 161/52 = 3.1$$

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

Limit of Quantification: It is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

Limit of Quantification for the drugs Abacavir, Lamivudine, and Zidovudine: The lowest concentration of the sample was prepared concerning the baseline noise and measured the signal to noise ratio. Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from the linearity curve by applying the following formula

$$\text{Limit of Quantification (LOQ)} = \sigma \times 10 / S$$

Where:
S – Slope of the calibration curve
σ – Residual standard deviation

Calculation of S/N Ratio for Abacavir: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOD solution (0.62% of target assay concentration): 524 µV

$$S/N = 524/52 = 10.0$$

Calculation of S/N Ratio for Lamivudine: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOQ solution (2.0% of target assay concentration): 518µV

$$S/N = 518/52 = 9.96$$

Calculation of S/N Ratio for Zidovudine: Average Baseline Noise obtained from Blank: 52 µV

Signal Obtained from LOQ solution (2.0% of target assay concentration): 527µV

$$S/N = 527/52 = 10.1$$

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution. The chromatograms were represented in **Fig. 15** and **16.**

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The standard and samples of Abacavir, Lamivudine, and Zidovudine were injected by changing the conditions of chromatography.

There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The Flow Rate was Varied at 0.4 ml/min to 0.6ml/min: The Standard solution of Abacavir, Lamivudine, and Zidovudine were prepared and analyzed using the varied flow rates along with method developed flow rate. On the evaluation of the above results, it was concluded that the variation in flow rate does not affect the method significantly. Hence, it was indicated that the method was robust even by a change in the flow rate. The chromatograms were represented in **Fig. 17** and **18, Table 13, 14** and **15**.

TABLE 13: RESULT FOR EFFECT OF VARIATION IN FLOW RATE FOR THE DRUG ABACAVIR

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	4160.3	1.4
2	0.25	3828.5	1.4
3	0.30	3896.0	1.4

TABLE 14: RESULT FOR EFFECT OF VARIATION IN FLOW RATE FOR THE DRUG LAMIVUDINE

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	3044.4	1.4
2	0.25	2842.6	1.4
3	0.30	2968.6	1.5

TABLE 15: RESULT FOR EFFECT OF VARIATION IN FLOW RATE FOR THE DRUG ZIDOVUDINE

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.20	6621.8	1.3
2	0.25	6452.0	1.3
3	0.30	6232.6	1.3

The Organic Composition in the Mobile Phase was varied from 65% to 55%: The Standard solution for the drug Abacavir, Lamivudine and Zidovudine were prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition. On the evaluation of the above results, it was concluded that the variation in 10%. Organic composition in the mobile phase does not affect the method significantly.

TABLE 16: IT SHOWS THE RESULT FOR EFFECT OF VARIATION IN MOBILE PHASE COMPOSITION FOR THE DRUG ABACAVIR (ORGANIC PHASE)

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3629.8	1.4
2	Actual	3828.5	1.4
3	10% more	3154.5	1.4

Hence, it was indicated that the method was robust even by change in the Mobile phase ±10. The chromatograms were represented in **Fig. 19** and **20**, **Table 16, 17** and **18**.

TABLE 17: IT SHOWS THE RESULT FOR EFFECT OF VARIATION IN MOBILE PHASE COMPOSITION FOR THE DRUG LAMIVUDINE (ORGANIC PHASE)

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2662.0	1.4
2	Actual	2842.6	1.4
3	10% more	2460.0	1.6

TABLE 18: IT SHOWS THE RESULT FOR EFFECT OF VARIATION IN MOBILE PHASE COMPOSITION FOR THE DRUG ZIDOVUDINE (ORGANIC PHASE)

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	7281.1	1.2
2	Actual	6452.0	1.3
3	10% more	4825.0	1.3

RESULTS AND DISCUSSION: The present work was undertaken to develop and validate a rapid and consistent UPLC method development in which the peaks will appear with a short period as per ICH Guidelines. The proposed method was a simple, fast, accurate, and precise method for the Quantification of the drug in the Pharmaceutical dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in the tablet dosage form.

The method was simple, precise, accurate, and sensitive and applicable for the simultaneous determination of Abacavir, Lamivudine, and Zidovudine in bulk drug and combined dosage forms. The Ultra performance liquid chromatography (UPLC) methods were developed and validated for simultaneous estimation of Abacavir, Lamivudine, and Zidovudine in bulk drug and combined dosage forms.

The UPLC separation was achieved on a Symmetry C_{18} (2.1 × 100mm, 1.7µm, Make: BEH) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (60%) whose pH was adjusted to 3.0 by using Ortho Phosphoric Acid & Methanol (40%) [UPLC Grade]. The flow

rate was monitored at 0.25 ml per min. The wavelength was selected for the detection was 280 nm. The run time was 3 min. The retention time found for the drugs Lamivudine, Abacavir, and Zidovudine was 1.019 min., 1.271 min. & 1.617 min. respectively. It was represented in **Fig. 4.** The Precision data for the drugs Abacavir, Lamivudine, and Zidovudine were represented in **Table 1, 2** and **3,** and the chromatograph was represented in **Fig. 6.** The % RSD for the sample should be NMT 2. The %RSD for the standard solution was found to be 0.14, 0.26 and 0.13 for the drugs Abacavir, Lamivudine, and Zidovudine respectively, which is within limits hence the method was precise.

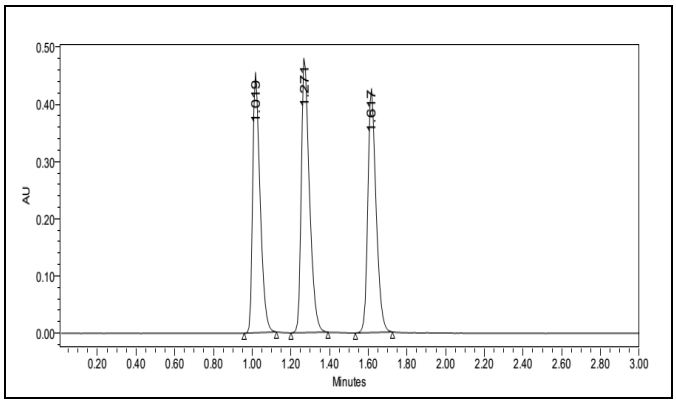

FIG. 4: CHROMATOGRAM FOR THE OPTIMIZED METHOD DEVELOPMENT

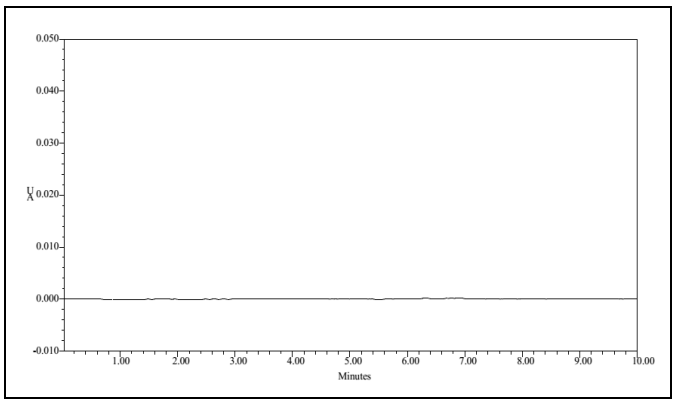

FIG. 5: CHROMATOGRAM FOR THE BLANK

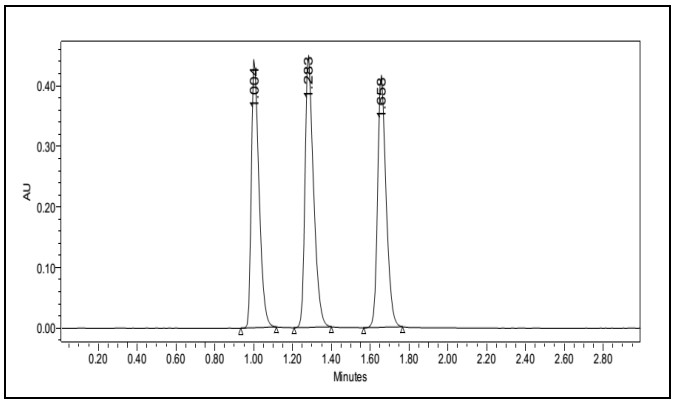

FIG. 6: CHROMATOGRAM FOR THE STANDARD DRUG

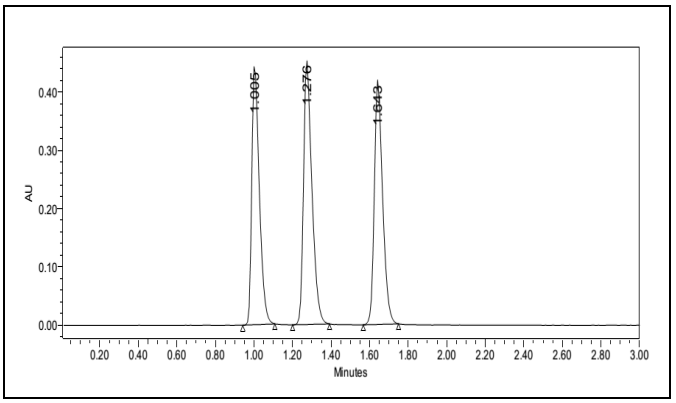

FIG. 7: CHROMATOGRAM FOR THE PRECISION

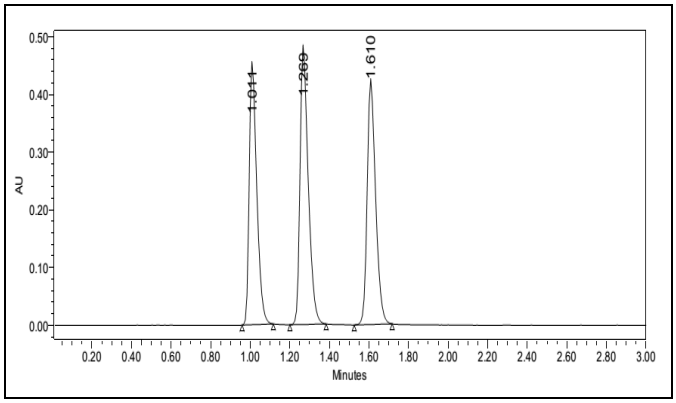

FIG. 8: CHROMATOGRAM FOR THE INTERMEDIATE PRECISION

When the drugs Abacavir, Lamivudine and Zidovudine were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in **Table 4, 5** and **6** and the chromatogram was represented in **Fig. 8**, which shows that the developed RP-HPLC method was highly precise. The % RSD was found to be 0.17, 0.18 & 0.21 for the drugs Abacavir, Lamivudine, and Zidovudine respectively, which is within limits.

The standard solution with Accuracy -80%, Accuracy -100% and Accuracy -120% was injected into the chromatographic system and calculated the amount found and amount added for Abacavir, Lamivudine, and Zidovudine and further calculated the individual recovery and mean recovery values. **Table 7, 8** and **9**.

The chromatograms were represented in **Fig. 9, 10** and **11**. The % recovery was found to be 98.0%-99.0% for the drug Abacavir. The % recovery was found to be 98.0% - 99.6% for the drug Lamivudine. The % recovery was found to be 98.2% - 98.6% for the drug Zidovudine.

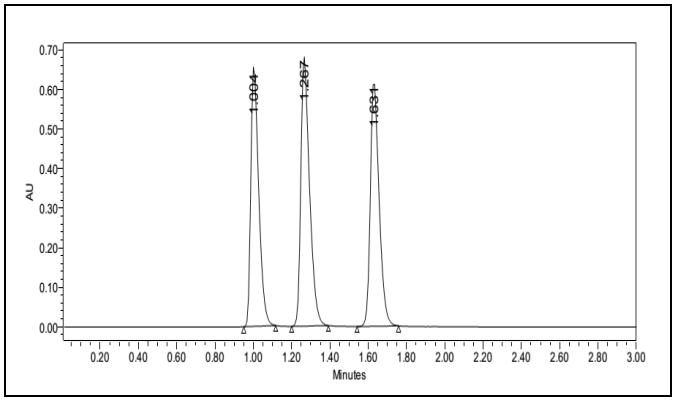

FIG. 9: CHROMATOGRAM FOR THE ACCURACY (80%)

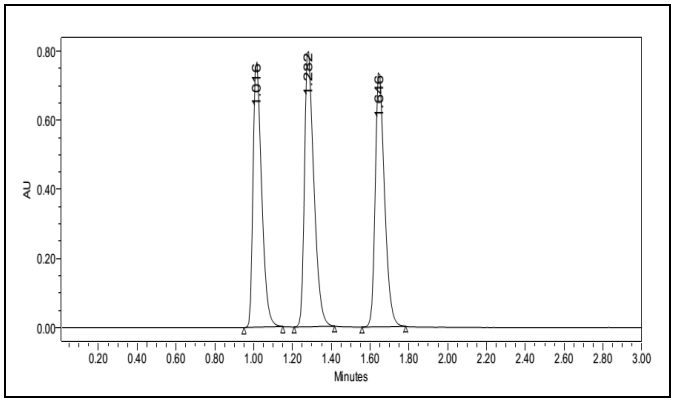

FIG. 10: CHROMATOGRAM FOR THE ACCURACY (100%)

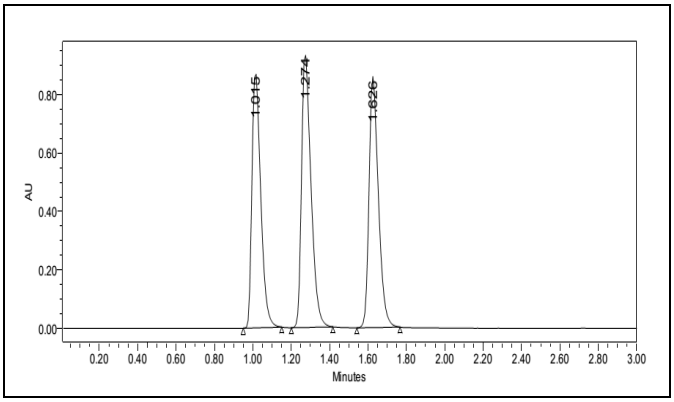

FIG. 11: CHROMATOGRAM FOR THE ACCURACY (120%)

To test the linearity of the method, five dilutions of the working standard solutions for the drugs Abacavir, Lamivudine, and Zidovudine were prepared. The linearity was established in the range of 20 to 60 ppm for the drug Abacavir & 10 to30ppm for the drug Lamivudine & 20 to 60 ppm for the drug Zidovudine. The data were represented in **Table 10, 11** and **12**. Each of the dilutions was injected into the column, and the Linearity Curve was represented in **Fig. 12, 13** and **14.** The Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999, which was in the acceptance limit.

The Limit of detection and limit of quantification of the method were calculated basing on the standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification.

The chromatograms were represented in **Fig 15** and **16**. The LOD for the drugs Abacavir, Lamivudine, and Zidovudine were found to be 0.002µg/ml, 0.003µg/ml, & 0.005µg/ml, respectively. The LOQ for the drugs Abacavir, Lamivudine, and Zidovudine were found to be 0.008 µg/ml, 0.01 µg/ml & 0.02 µg/ml respectively. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution**.** The results obtained were within the limit. The Robustness of the method was found out by testing the effect of small, deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate Buffer and Methanol [HPLC Grade] in the mobile phase.

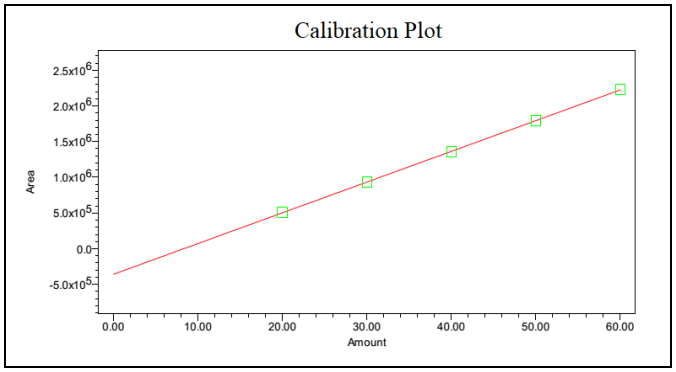

FIG. 12: LINEARITY CURVE FOR THE DRUG ABACAVIR

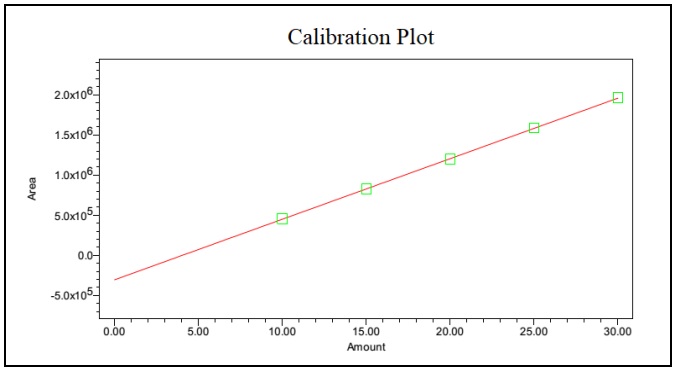

FIG. 13: LINEARITY CURVE FOR THE DRUG LAMIVUDINE

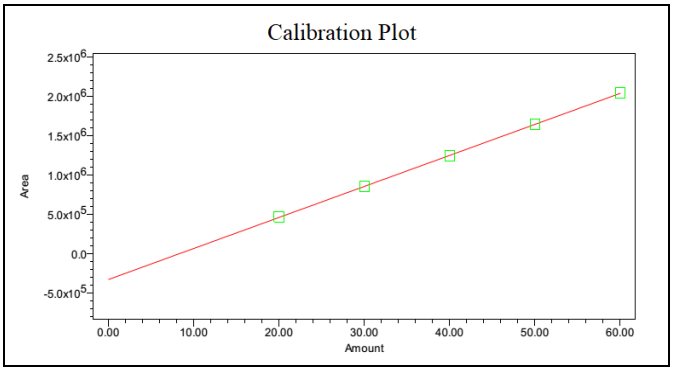

FIG. 14: LINEARITY CURVE FOR THE DRUG ZIDOVUDINE

The method was found to be robust enough that the peak area was not affected by a small variation in the chromatographic conditions. The system suitability parameters were within limits and shown in **Table 13, 14, 15, 16, 17** and **18,** and chromatograms were represented in **Fig. 17, 18, 19** and **20**.

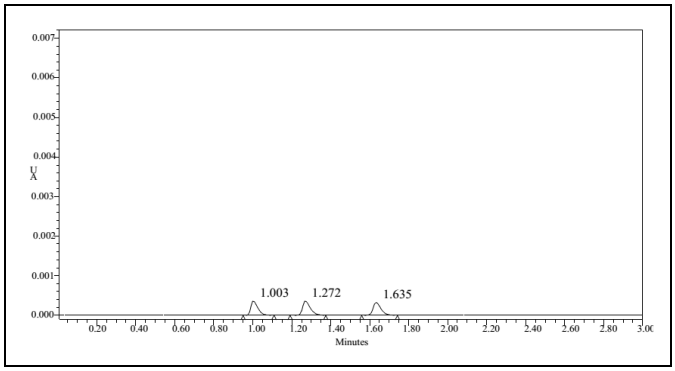

FIG. 15: CHROMATOGRAM FOR THE DRUG ABACAVIR, LAMIVUDINE & ZIDOVUDINE (LOD)

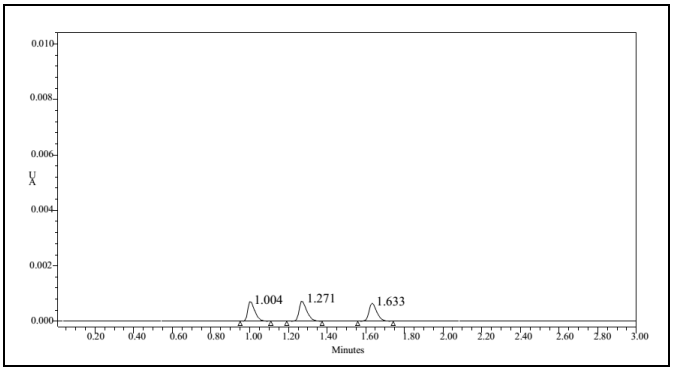

FIG. 16: CHROMATOGRAM FOR THE DRUG ABACAVIR, LAMIVUDINE & ZIDOVUDINE (LOQ)

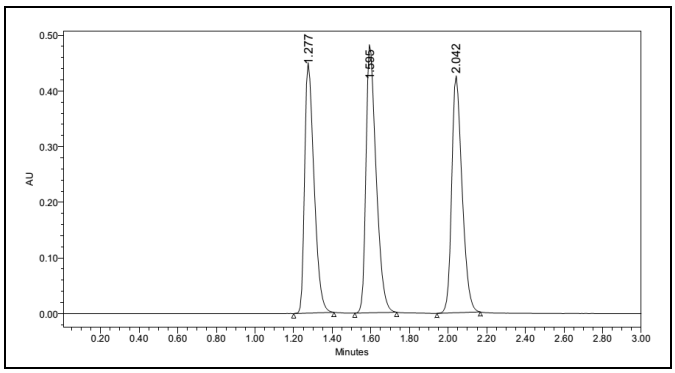

FIG. 17: CHROMATOGRAM FOR LESS FLOW RATE (0.2ml/min)

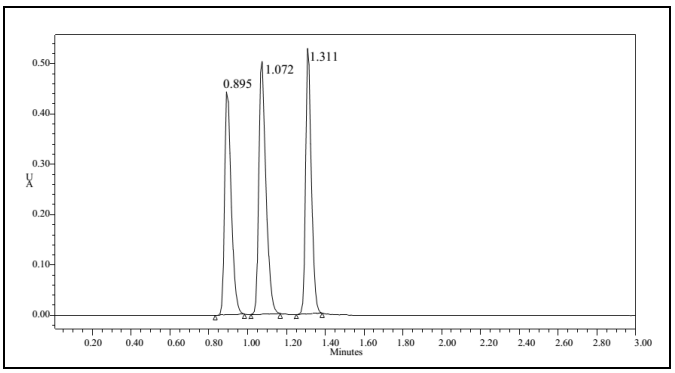

FIG. 18: CHROMATOGRAM FOR MORE FLOW RATE (0.3ml/min)

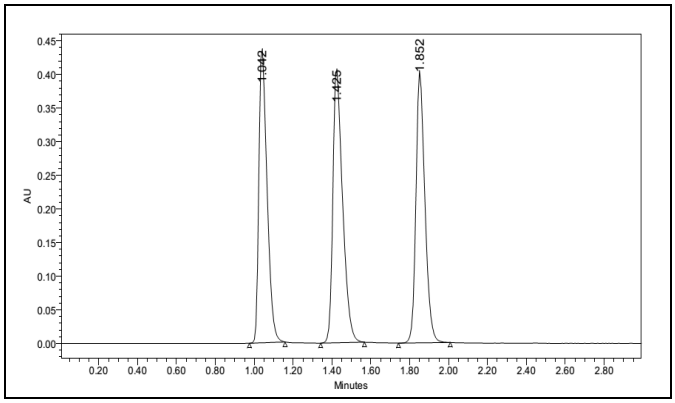

FIG. 19: CHROMATOGRAM FOR LESS ORGANIC COMPOSITION (55%)

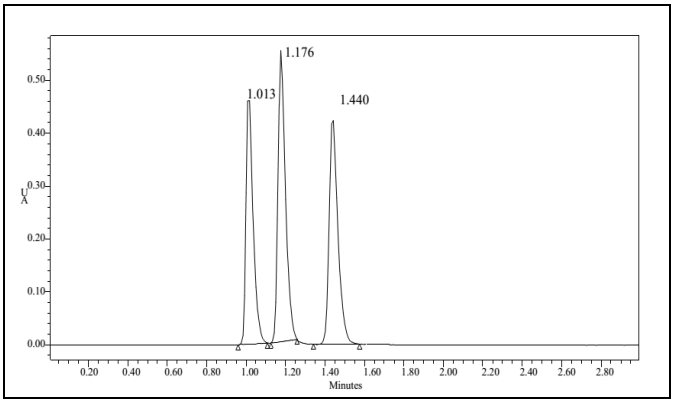

FIG. 20: CHROMATOGRAM FOR MORE ORGANIC COMPOSITION (65%)

CONCLUSION: Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities.

In industry, the quality assurance and quality control departments play a major role in bringing out a safe and effective drug or dosage form.

The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist on the adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision, and accuracy in the estimation of drugs. It was concluded that the proposed new UPLC method developed for the quantitative determination of Abacavir, Lamivudine, and Zidovudine in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical.

The sample recoveries in the formulation were in good agreement with their respective label claims, and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Abacavir, Lamivudine, and Zidovudine depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological, and pharmacokinetic studies for the drug Abacavir, Lamivudine and Zidovudine. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases.

FUTURE ASPECT: The proposed method can be used in the future for the clinical, biological, and pharmacokinetic studies of Abacavir, Lamivudine, and Zidovudine.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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