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Simultaneous Quantification of Lamivudine, Zidovudine and Related Impurities in Fixed Oral Dosage Combination Using RP-HPLC with DAD detection

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Abstract

A simple and fast isocratic Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed and validated for the simultaneous determination of Lamivudine, zidovudine and their related impurities in tablets. The method consists of a mobile phase combination of Acetonitrile (HPLC grade) and Buffer (0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 ml of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid to a final volume preparation of 100 ml) in the ratio 10:90. Phenomenex Luna 5-µm C18 (2)-250 x 4.6-mm, 5-µm) was used as the stationary phase. The column oven was set to a temperature of 30±1^{o}C. Quantification was achieved with a DAD detector set at 270 nm.

Resolution was achieved at a short run time of 25 minutes. Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine and Zidovudine related impurity B eluted at 3.749±0.004, 4.862±0.013, 15.332±0.064, 21.201±0.076 and 23.682±0.117 respectively. Relative retention times (RRT) for lamivudine unknown related impurities with respect to Zidovudine were 0.15, 0.17, 0.30 and 0.59. RRT for Zidovudine unknown related impurities with respect to Zidovudine were 0.39 and 0.63. The method was found to be specific, robust, accurate and precise for the estimation of Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine and Zidovudine related impurity B in fixed oral dosage tablets over the concentration ranges of 0.0204 mg/mL-0.0088 mg/mL, 0.0962 mg/mL-0.7699 mg/mL, 0.1929 mg/mL-1.5410 mg/mL and 0.0088 mg/mL-0.024 mg/mL respectively. The Correlation Coefficient (r^2) for Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine and Zidovudine related impurity B were greater than 0.998. The LOD were found to be between $1.9x10^{-4}$ mg/mL to 2.69 $x10^{-4}$ mg/mL. The proposed method is precise, specific, accurate and robust for the simultaneous estimation of Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine, Zidovudine related impurity B and other related impurities in dosage forms.

Keywords: Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine, Zidovudine related impurity B, Lamivudine related impurities, Zidovudine related impurities RP-HPLC, Validation.

1. INTRODUCTION

Lamivudine and Zidovudine are synthetic nucleoside analogues with activity against HIV-1. Lamivudine and Zidovudine are two nucleoside analogue reverse transcriptase inhibitors. Intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP is inhibition of reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue. 3TC-TP is a weak inhibitor of cellular DNA polymerases α, β, and γ.[1] It has a molecular formula of $C_8H_{11}N_3O_3S$ with a molecular mass 229.26 g/mol.

Zidovudine once absorbed is phosphorylated to its active 5′-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). The principal mode of action of ZDV-TP is inhibition of RT via DNA chain termination after incorporation of the nucleotide analogue. ZDV-TP is a weak inhibitor of the cellular DNA polymerases α and γ and has been reported to be incorporated into the DNA of cells in culture. [1]

Its molecular formula is, $C_{10}H_{13}N_5O_4$ with a molecular mass of 267.24 g

Few chromatographic methods for the simultaneous determination of lamivudine, Zidovudine and related impurities have been reported. Few methods available includes those in the compendia. [2,3] The aim of this study is to develop a simple RP-HPLC method in a model tablet formulation containing 150 mg of Lamivudine and

300 mg of zidovudine. This study was done in accordance with the International Conference on Harmonization (ICH) guidelines [4,5,6,7,8].

2. MATERIALS AND METHODS

2.1 Materials and Reagents:

Chemicals / Reagents: Acetonitrile (Manufacturer: Fisher Scientific, Batch #: 0803950), Hydrochloric Acid (BN: H1/65/450-1, Manufacturer: M&B, Purity: 36 %w/w), 2 M sodium hydroxide, 35 % hydrogen peroxide, USP Purified Water and Doubly distilled water.

Analytical Reference Standards: USP Lamivudine (Lot #: H0H087, Potency: 99.6 %), USP Zidovudine RS (Lot #: HOF263, Potency: 99.0 %), USP Zidovudine Related Compound B RS (Lot #: H0F230, Potency: 100.0 %), USP Zidovudine Related Compound C RS (Lot #: GOG181, Potency: 100.0 %), Salicylic acid (Lot #: KOF112), Potency: 99.8 %). Lamivudine Working standard (Manufacturer: Shanghai Desano Chemical Co. LTD. China, Lot #: DH010-4-090405, Potency (OAB): 98.34 %. Zidovudine Working Standard (Manufacturer: Shanghia Desano Chemical CO Ltd, Lot #: DH006-4-090430, Potency (OAB): 100.93 %.

Pharmaceutical Excipients: Microcrystalline cellulose (Batch #: 0028, Manufacturer: Brahmar Cellulose Products PVT Ltd, Solutab (Croscamellose Sodium) (Batch #: 8199/09, Manufacturer: Blanver Farmoquimica Ltd, Magnesium Stereate (Batch #: MGSV80097, Manufacturer: Stockbridge International Ltd. Aerosil (Colloidal Silicon Dioxide) (Batch #: 132700021, Manufacturer: Biochemie.

2.2 Instrumentation and Chromatographic conditions

Agilent Technologies 1200 series HPLC modules (G1315D, G1315A, G1329A, G1311A, G1332A and organizer, with ChemStation data processing software), Sonicator, OHAUS Analytical Balance. Stainless steel column 250 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (Phenomenex Luna 5-µm C18 (2)-250 x 4.6-mm, 5-µm). Mobile phase composition was 90(buffer): 10 Acetonitrile. Buffer was prepared from 0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 ml of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid diluted with distilled water to a final volume of 100 ml. The mobile phase was pumped through the column at a flow rate of 1 mL/minute. The sample injection volume was 10 µL. The UV detector was set at a wavelength of 270 nm for the detection. Column oven was set at 30±1 ^{o}C and the run time was 25 minutes.

2.3 Tablet formulation

Tablets were prepared by compression after wet granulation with Clit compression machine (Chamunda Pharma Machinery Private Limited, Ahmedabad, Gujarat; India). Model tablets containing 150 mg lamivudine and 300 mg zidovudine. Microcrystalline cellulose, Solutab (Croscamellose Sodium), Magnesium Stereate and Aerosil (Colloidal Silicon Dioxide) were the excepients.

Samples were analysed using the developed RP-HPLC method. The results were reported as means ± S.D (standard error of the mean). Results were statistically analysed for significant differences.

2.4 Validation Parameters

The validation exercise was performed as per ICH guidelines. It is applicable for the analysis of Lamivudine, Zidovudine and related impurities in combined oral dosage forms.

Specificity: This was performed by injecting 10 µL aliquot of the Mobile phase (diluent), Placebo solution, diluent spiked separately with zidovudine related impurity C standard, Lamivudine working standard, Lamivudine salicylic acid, Zidovudine working standard, and Zidovudine related impurity B working standard..

System suitability test was determined by making six replicate injections of the standard solution. The respective peak responses and the RSD for six replicate injections were recorded. The retention time, RSD of relative peak areas were recorded and are represented in Table 1. A sample chromatograms are shown in Figure 1 to 5.

Forced degradation and Selectivity: lamivudine, zidovudine and placebo were incubated separately in 2M HCl, 2M NaOH, 30 % H_2O_2 and at a temperature of 105^{oC} for a period of 48 hrs. 10 µL aliquot of the product were injected. Each degradant was tagged as related impurities to the mother API from which they were generated.

Mixture of the degradation product spiked with known USP related impurity standards were also analysed. Relative retention times of each peaks was observed relative to zidovudine in the degraded mixture.

The precisions were determined by evaluating Repeatability (intraday precision) and Intermediate precision (interday precision). Intraday precision was determined by preparation and analysis of the same sample (all known standards and placebo) in six replicates. This was evaluated at various time points (0 (initial), 4, 8,12, 16, 20 and 24 hours) by the same analyst. Interday precision was performed in six replicates at intervals of one day by two different analysts over a period of six days. The Percentage contents and relative standard deviation (RSD) were determined in each case. The results were subjected to statistical analysis at 95 % confidence interval to determine any significant differences.

Accuracy was determined using the method of spiking. Six different amounts corresponding to 80 %, 100 % and 120 % concentrations of zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine, and Zidovudine related impurity B working standard and placebo were analysed. The nominal concentrations were compared to the actual concentrations and the percentage recoveries were noted. The results were subjected to statistical analysis at 95% confidence interval to determine any significant differences.

The stability of mixture of zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine, Zidovudine related impurity B working standard and placebo all dissolved in the diluent were evaluated. The prepared solution was kept at ambient temperature (25^{o}C±2 ^{o}C) and analyzed at various time points (0 (initial), 3, 6, 9, 24 hours). The responses were compared with those of freshly prepared solution and analysed statistically.

Linearity and Working Concentration range, LOD and LOQ were determine for Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine and Zidovudine related impurity B.

Robustness was determined by making deliberate changes to the following parameters; flow rate, mobile phase composition and the pH of the mobile phase. Temperature and column were also changed. Results obtained were subjected to statistical analysis.

2.5 Application of Proposed Method for the Analysis of Tablet Formulation

Twenty tablets were weighed, the average weight was noted and powdered. Samples of the powdered tablet equivalent to 48 mg of lamivudine and 98 mg of zidovudine were weighed and transferred into a 100 mL volumetric flask. 60 mL of the diluent was added and sample sonicated for 5 minutes. Solution was made to volume with the diluent.

The standard solution was prepared by weighing 48.5 mg of Lamivudine and 97.9 mg of zodovudine working standards into a 100 mL volumetric flask. 60 mL of the diluent was added and sample sonicated for 5 minutes. Solution was made to volume with the diluent

Approximaly 0.0124 mg/mL of Zidovudine related compound C, lamivudine salicylic acid and Zidovudine related compound was prepared for the analysis.

The peak areas in the tablet samples were compared to that of the standard and their percentage contents recorded.

3. RESULTS AND DISCUSSION

To develop a relatively simple system, Acetonitrile (HPLC grade) and Triethylamine were used as the only organic solvent with buffer (Buffer (0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 mL of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid to a final volume preparation of 100 mL)

Various mobile phase acetonitrile to buffer in the ratios 40:60, 20:80 and 10:90 were used. Mobile composition which gave better resolution was observed to be 10(Acetonitrile):80 (buffer).

Phenomenex Luna 5-µm C18 (2)-(250 x 4.6-mm, 5-µm) as the stationary phase was adopted and used for the analysis.

A wave length of 270 nm was adopted for appreciable peak area for the analyte. A flow rate of 1mL/min, run time of 25 minutes, injection volume of 10 µL and a column oven temperature of 30 ^{o}C were adopted for the analysis.

3.1 Method Development

3.2 Method Validation

In evaluating specificity, there were no interferences. Injections of the placebo and the mobile phase (diluent) gave no peaks. Mean retention times are shown in table 1.

Table 1: Results of specificity

No	Solution	Mean retention time (minutes)
1	Diluent	no peak
2	Placebo	no peak
3	Zidovudine related impurity C	3.769±0.004
4	Lamivudine	4.855±0.013
5	Lamivudine salicylic acid	15.532±0.064
6	Zidovudine	21.001±0.076
7	Zidovudine Related impurity B	23.282+0.011

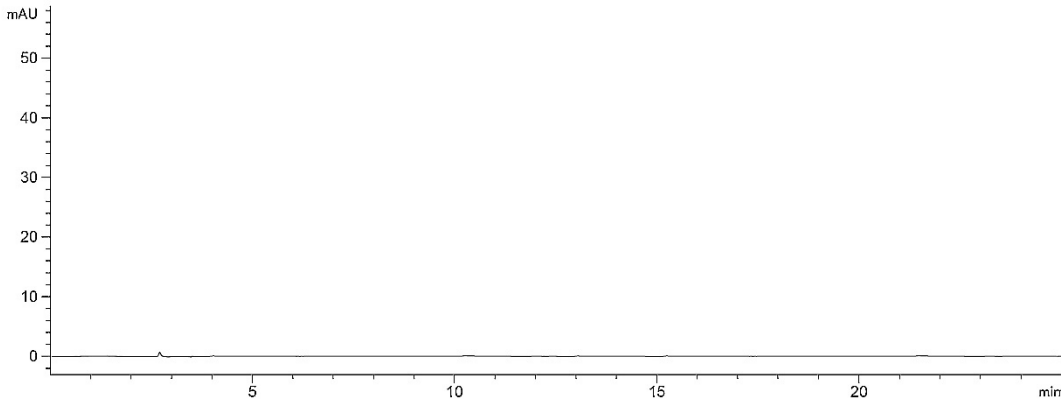

Fig. 1: Sample Chromatogram of blank Injection

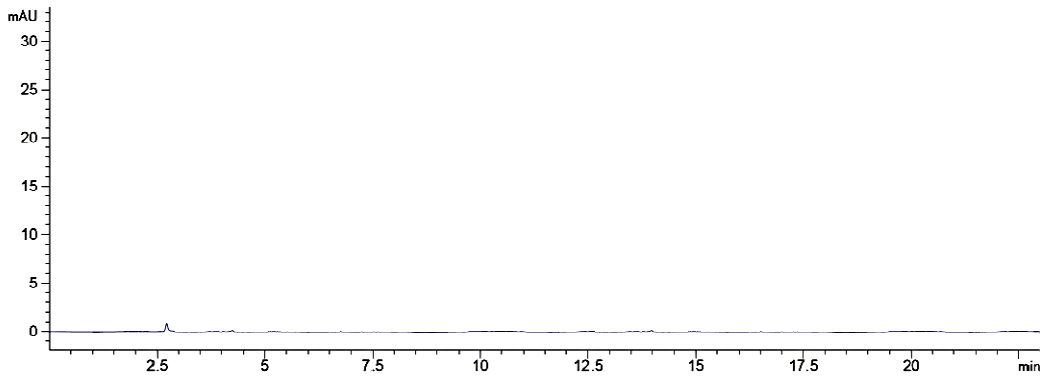

Fig. 2: Sample Chromatogram of placebo Injection

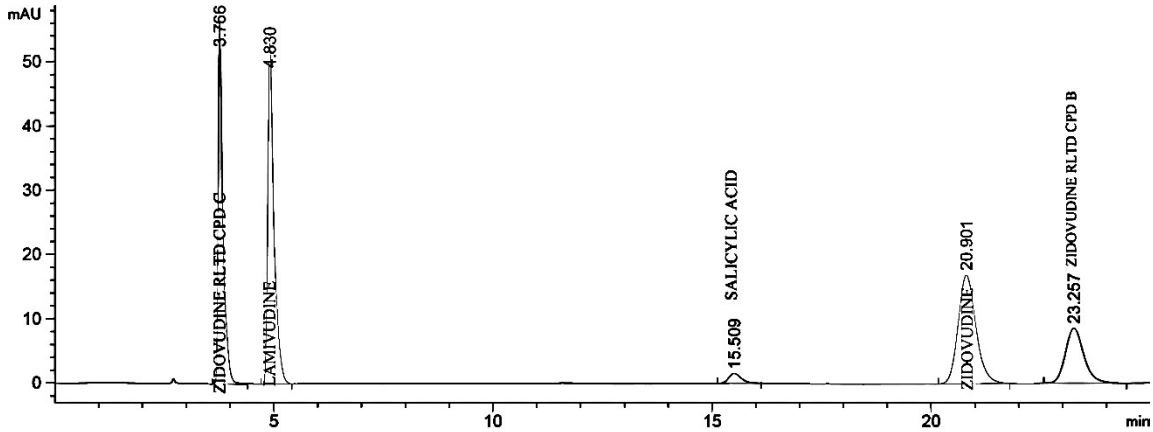

Fig. 3: Sample Chromatogram of placebo spiked with API and know related impurities

In evaluating the degraded product and selectivity of the method, there were no interferences from unidentified peaks formed during the degradation process. . Injections of the placebo and the mobile phase (diluent) gave no peaks. The relative retention time (RRT) for each peak was calculate relative to zidovudine in the degraded standard mixed placebo spiked related impurity mixture. Refer to table 2 for relative retention times and Fig 4 and Fig 5 for chromatograms.

Table 2: Relative retention times for known and unknown compounds

No	**Name**	**RRT**
1	Lamivudine Related Compound	0.15
2	Lamivudine Related Compound	0.17
3	Zidovudine Related Compound C	0.18
4	**Lamivudine**	0.23
5	Lamivudine Related Compound	0.30
6	Zidovudine Related Compound	0.39
7	Lamivudine Related Compound	0.59
8	Zidovudine Related Compound	0.63
9	Lamivudine Salicylic acid	0.74
10	**Zidovudine**	1.00
11	Zidovudine Related Compound B	1.11

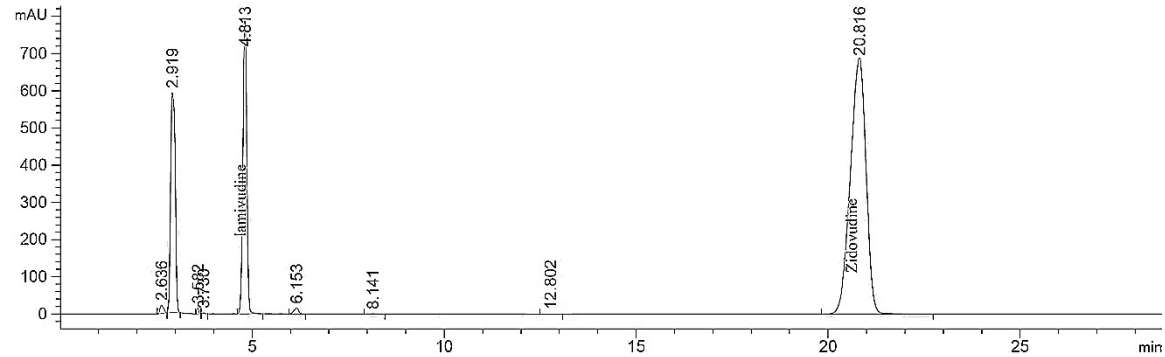

Fig. 4: Sample Chromatogram of degraded mixture of placebo, lamivudine and zidovudine

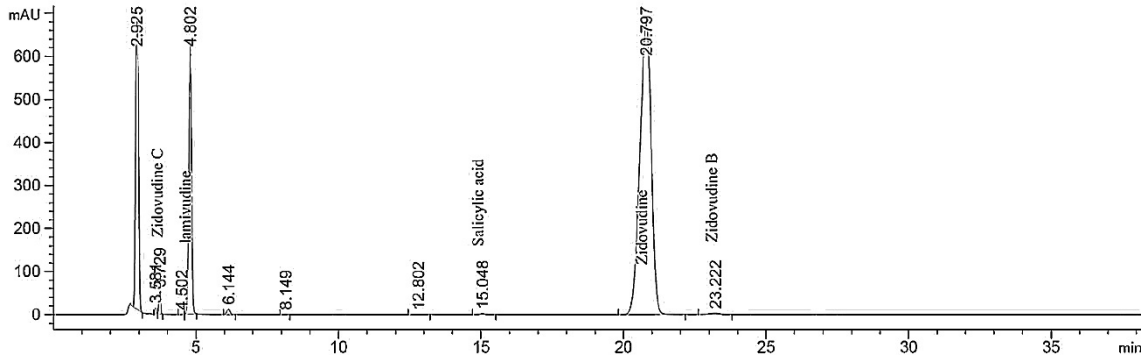

Fig. 5: Sample Chromatogram of degraded mixture of placebo, lamivudine and zidovudine spiked with USP related compound standard.

The % RSD obtained for Repeatability and reproducibility using the four known standards with placebo were in the range of 0.15 - 0.80 and 0.01- 0.44 respectively. The method is of high precision since % RSD were less than 1.00 %.

Table 3: Repeatability (Intraday precision) over study period of 24 Hours

	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Mean Recovery	100.25±0.15	100.51±0.81	100.13±0.34	99.82 ± 0.50	99.64±0.39
% RSD	0.15	0.80	0.34	0.51	0.39

Table 4: Reproducibility (intermediate precision) by First analyst over a period of Six days.

	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Mean Recovery	100.21±0.35	99.43±0.43	99.95±0.33	100.29 ± 0.50	99.83±0.17
% RSD	0.25	0.44	0.33	0.5	0.17

Table 5: Reproducibility (intermediate precision) by second analyst over a period of Six days.

	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Mean Recovery	100.11±0.23	99.41±0.14	99.99±0.01	100.09 ± 0.70	100.01±0.04
% RSD	0.23	0.14	0.01	0.7	0.04

Analysis of variance (ANOVA) performed at 95 % confidence interval reveals that there were no statistically reliable difference between the amounts recovered for the various active compounds analysed during the intraday and intermediate precision testing. P value observed were greater than 0.05, ($p > 0.05$).

The accuracy of the recovery studies proves the quantification of target compounds within the acceptable limits. The percentage recovery were found to be in the range of 99.58 % - 100.53 %. Analysis of variance performed at 95 % confidence interval reveals that there were no significant differences between the results obtained at the various concentration levels. P value observed were greater than 0.05, ($p > 0.05$).

Table 6: Mean Recovery at different concentration level.

Mean Recovery	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
80%	100.01±0.12	99.89±0.11	99.99±0.14	100.13 ± 0.21	100.11±0.11
100%	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
120%	100.21±0.35	100.32±0.13	100.03±0.12	99.58 ± 0.10	100.53±0.21

Stability studies of solution containing mixture of zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine, Zidovudine related impurity B working standard and placebo were studied within a period of 24 hours. Statistical analysis performed on each analyte after the 24 hour period shows that there were no statistical reliable difference in the recovery of sample at 95 % confidence interval. P value obtained in each case were > 0.05.

Table 7: Results of % Stable analyte in sample solution over a study period of 24 Hours

storage time(hours)	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
0	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
4	99.99±0.26	100.0±0.31	99.10±0.23	99.99 ± 0.54	99.99±0.32
8	100.09±0.12	98.95±0.23	99.00±0.41	100.0 ± 0.62	99.45±0.13
12	100.11±0.03	99.95±0.34	98.03±0.52	99.67 ± 0.54	99.58±0.10
16	98.9±0.63	99.45±0.14	98.00±0.82	99.12 ± 0.34	99.41±0.64
24	99.9±0.63	99.45±0.14	98.00±0.82	99.98 ± 0.10	99.42±0.23

Linearity and Working Concentration range, LOD and LOQ were determine for Zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine and Zidovudine related impurity B. The working concentration ranges from 0.0088 mg/mL to 0.0204 mg/mL for Zidovudine related impurity C, 0.0962 mg/mL to 0.7699 mg/mL for Lamivudine, 0.0087 mg/mL to 0.0204 mg/mL for Lamivudine salicylic acid, 0.1927 mg/mL to 1.5410 mg/mL for zidovudine and 0.0088 mg/mL to 0.0204 mg/mL for Zidovudine related impurity B. The Correlation co-efficient were observed to be 0.9975, 0.9997, 0.9983, 0.9991 and 0.9999 respectively. With regards to residual plot, the residuals were randomly dispersed.

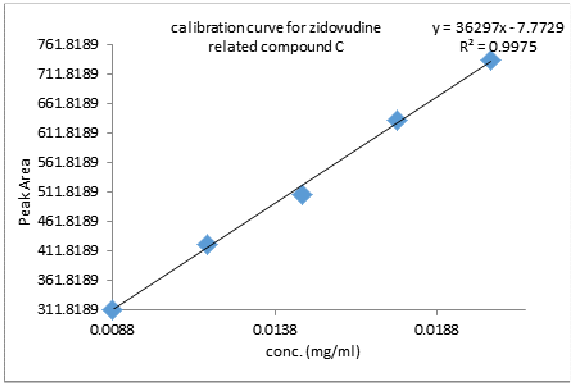

Fig. 6: Calibration curve for zidovudine related compound C

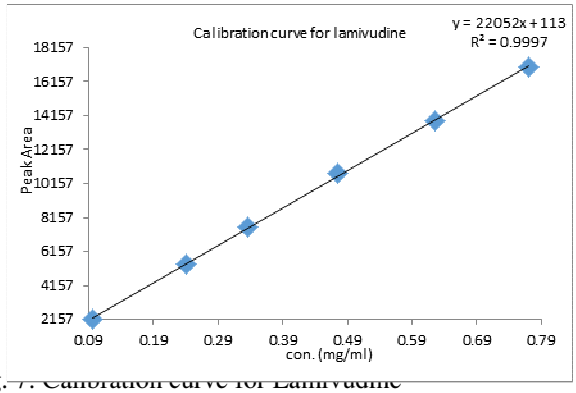

Fig. 7: Calibration curve for Lamivudine

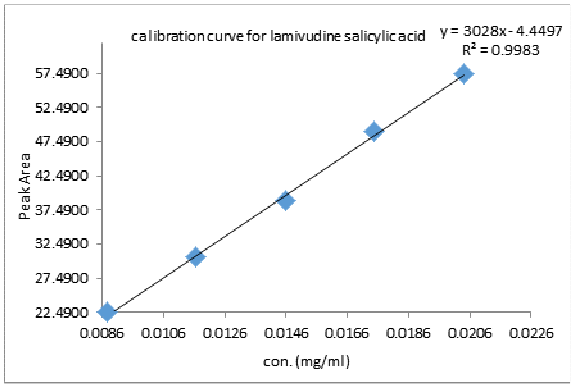

Fig 8: Calibration curve for lamivudine salicylic acid

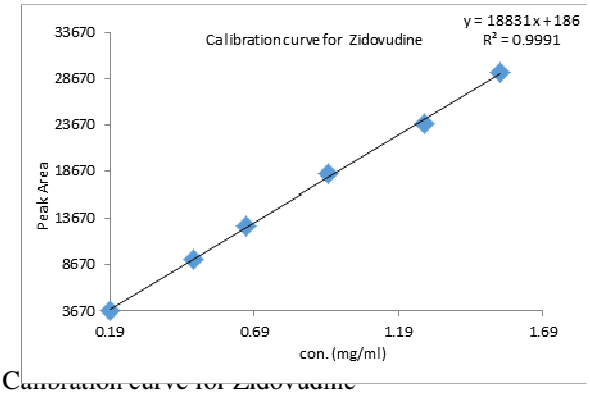

Fig 9: Calibration curve for Zidovudine

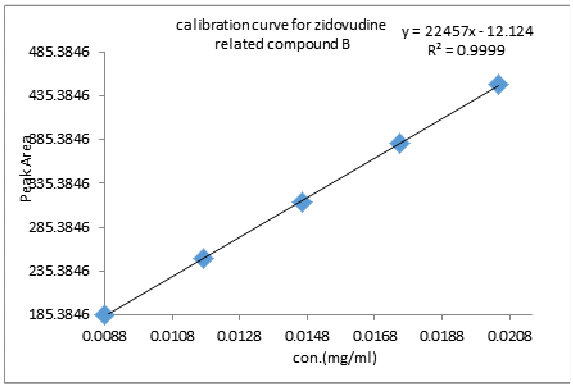

Fig 10: Calibration curve for Zidovudine related compound B

Table 8: Linear regression data, LOQ and LOD for calibration curves

API	Zidovudine related compound C	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Concentration range (mg/mL)	0.0088 to 0.0204	0.0962 to 0.7699	0.0087 to 0.0204	0.1927 to 1.5410	0.0088 to 0.0204
slope	36297	22052	3028	18831	22457
Intercept	-7.7729	113	-4.4497	186	-12.124
Correlation coefficient	0.9975	0.9997	0.9983	0.9991	0.9999
LOD(mg/mL)	1.12E-04	1.80E-04	1.44E-03	2.69E-04	1.67E-04
LOQ(mg/mL)	3.40E-04	5.45E-04	4.36E-03	8.15E-04	5.07E-04

With deliberate changes made to flow rate, pH of Mobile phase composition and temperature, the % RSD were observed to be in the range of 0.01 – 0.92.

Column deliberately changed to 10 µm could not resolve all the test standards due to overlap of peaks. However, lamivudine and zidovudine were well resolved. Phenomenex Luna C18 (2) 250 x 4.6-mm, 10-µm) can therefore not be used for routine analysis of Zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine and Zidovudine related impurity B.

Deliberate change of flow rate from 1.0 minutes to 2.0 minutes using Phenomenex Luna C18 (2) 250 x 4.6-mm, 10-µm reduced the analysis time of lamivudine and zidovudine from 8 minutes to 4.0 minutes. Refer to fig: 11 and Fig 12.

Table 9: Changed factors and recovery for Robustness of method.

Change Factors	level	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Flow Rate						
0.8 mL/min	-2	99.95±0.21	99.34±0.33	100.22±0.31	99.34 ± 0.53	99.21±0.23
1.0 mL/min	0	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
1.2 mL/min	2	99.45±0.53	100.31±0.32	99.90±0.62	100.32 ± 0.51	100.04±0.11
pH						
7.8	-2	99.30±0.31	100.23±0.13	100.01±0.01	99.12 ± 0.43	99.31±0.43
8	0	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
8.2	2	99.71±0.33	100.01±0.12	99.21±0.65	100.04 ± 0.42	100.23±0.32
Mobile phase composition						
Buffer (pH 8.0):Acetonitrile	88:12	99.32±0.71	100.01±0.45	100.51±0.65	99.98 ± 0.6.7	99.80±0.45
Buffer(pH 8.0):Acetonitrile	90:10	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
Buffer(pH 8.0):Acetonitrile	92:8	99.98 ±0.31	100.45±0.43	100.54±0.32	99.43 ± 0.54	99.41±0.92
Temperature						
25 ^{O}C	-5	99.82±0.54	99.91±0.34	100.04±0.52	99.78 ± 0.47	99.89±0.11
30 ^{O}C	0	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
35 ^{O}C	5	99.78±0.65	99.91±0.54	99.97±0.15	99.45 ± 0.78	98.98±0.34

Table 9: Continuation of Changed factors and recovery for Robustness of method

Change Factors	level	Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
column						
Phenomenex Luna C18 (2)	250 x 4.6-mm, 5-µm)	99.91±0.22	99.91±0.21	100.10±0.12	99.95 ± 0.42	99.87±0.21
Phenomenex Luna C18 (2)	250 x 4.6-mm, 10-µm)	-	99.83±0.67	-	99.98 ± 0.89	-

Table 10: Elution time at different flow rate with different columns.

	Lamivudine		Zidovudine	
	1.0 mL/min	2.0 mL/min	1.0 mL/min	2.0 mL/min
Phenomenex Luna C18 (2) 250 x 4.6-mm, 5- µm)	4.855±0.013	-	21.001±0.076	-
Phenomenex Luna C18 (2) 250 x 4.6-mm, 10-µm)	2.821±0.023	1.790±0.067	7.432±0.056	4.782±0.176

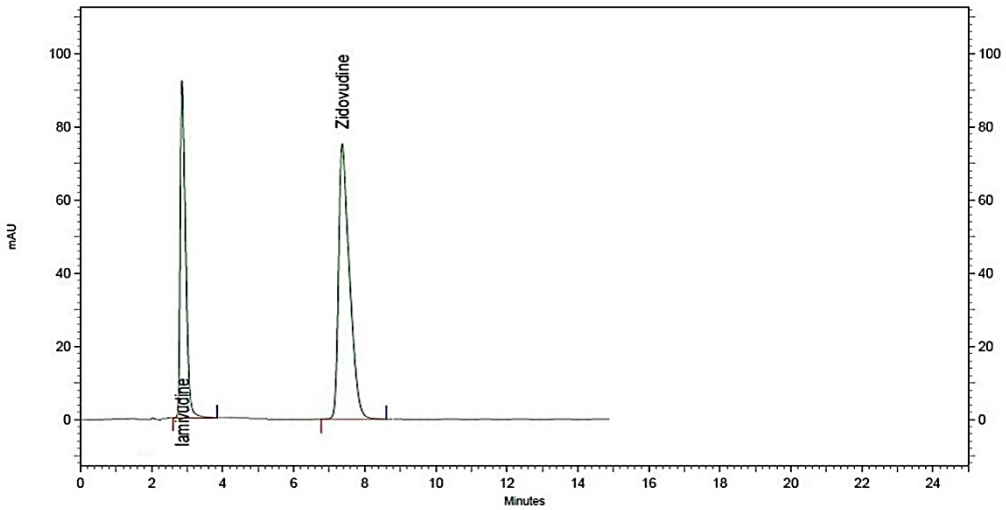

Fig. 11: Sample Chromatogram of lamivudine and zidovudine using Phenomenex Luna 10-µm C18 (2)-250 x 4.6-mm, 10-µm at a flow rate of 1.0 mL/min.

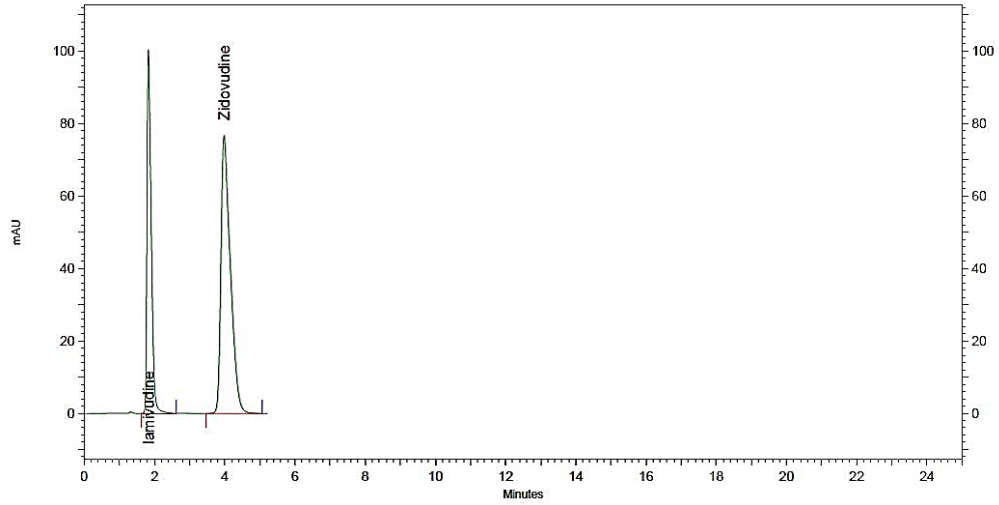

Fig. 12: Sample Chromatogram of lamivudine and zidovudine using Phenomenex Luna 10-µm C18 (2)-250 x 4.6-mm, 10-µm at a flow rate of 2.0 mL/min.

The mean recovery of Zidovudine related compound c, Lamivudine, Lamivudine Salicylic acid, Zidovudine and Zidovudine related compound B in the formulated tablet were quantified as per the method developed. Refer to table 11 for percentage recovery.

Table 11: Mean Percentage recovery of lamivudine, zidovudine and known related impurities in formulated tablet.

Zidovudine related compound c	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
0.0123±0.000133	99.87±0.23	0.00341±0.000014	100.01 ± 0.34	0.0045 ± 0.000012

4. CONCLUSION

The proposed RP-HPLC method for the estimation of Zidovudine related compound c, Lamivudine, Lamivudine Salicylic acid, Zidovudine and Zidovudine related compound B in model tablets and API was validated as per ICH guidelines. The method was found to be specific, robust, accurate and precise for the estimation of Zidovudine related compound c, Lamivudine, Lamivudine Salicylic acid, Zidovudine and Zidovudine related compound B in the fixed oral dosage tablets over the concentration ranges of 0.0204 mg/mL-0.0088mg/mL, 0.0962 mg/mL-0.7699 mg/mL, 0.1929 mg/mL-1.5410 mg/mL and 0.0088 mg/mL-0.024 mg/mL respectively. LOD in order of the above stated active compounds were observed to be $1.12x10^{-4}$ mg/mL, $1.8\ x10^{-4}$ mg/mL, $1.44\ x10^{-3}$ mg/mL, $2.69\ x10^{-4}$ mg/mL and $1.67\ x10^{-4}$ mg/mL. LOQ in the same order were $3.40x10^{-4}$ mg/mL, $5.45\ x10^{-4}$ mg/mL, $4.36\ x10^{-3}$ mg/mL, $8.15x10^{-4}$ mg/mL and $5.07x10^{-4}$ mg/mL.

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