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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF ZIDOVUDINE AND LAMIVUDINE IN FIXED-DOSE TABLET FORMULATIONS: DEVELOPMENT, OPTIMIZATION AND VALIDATION AS PER ICH GUIDELINES

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ABSTRACT:

The simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) novel method was developed and evaluated for the estimation of Zidovudine and Lamivudine in the tablet dosage form. Chromatographic separation was achieved using methanol: water (50:50 v/v) was A BDS hypersin C-18 (150×4.6×5µ) column, at oven temperature of 25 degrees had been utilised .The flow rate was 0.6ml/min maintained and effluents were monitored at 281 nm. Zidovudine and Lamivudine were observed to elute at retention times of 4.6 minutes and 2.6 minutes, respectively, in the chromatographic analysis. The linearity, accuracy, precision, limit of detection, limit of

quantification and robustness of the method was validated.The calculated correlation coefficient (r^2) was 0.999.The percentage recovery values were found to be 100.0% and 100.3%, while the %RSD for precision was 0.2 for both measurements. The purity of the samples was estimated to be 99.9% w/w for each. The recovery values for Lamivudine and Zidovudine were within the established acceptable range. Proposed and evaluated method can be successfully applied for the quantitative determination of Zidovudine and Lamivudine in combined pharmaceutical dosage forms.

KEYWORDS: Antiretroviral drug analysis, Zidovudine–Lamivudine combination, RP-HPLC method validation, Pharmaceutical quality control.

INTRODUCTION:

Combination antiretroviral therapy (cART) has significantly improved the management of human immunodeficiency virus (HIV) infection by reducing viral load and improving patient survival rates [1,2]. Among the commonly prescribed fixed-dose combinations, Zidovudine and Lamivudine are widely used due to their synergistic antiviral activity and favorable safety profile [3].

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) that undergoes intracellular phosphorylation to its active triphosphate form, which inhibits viral DNA synthesis by causing premature chain termination [4]. Similarly, Zidovudine is metabolized to carbovir triphosphate, which competitively inhibits HIV reverse transcriptase and prevents viral replication [5].

Ensuring the quality and consistency of such combination formulations is essential for therapeutic efficacy and patient safety. Reverse phase high performance liquid chromatography (RP-HPLC) is widely employed for the simultaneous estimation of pharmaceutical compounds due to its high sensitivity, accuracy, and reproducibility [6,7].

Method validation is a critical step in pharmaceutical analysis to confirm that an analytical method is suitable for its intended purpose. According to ICH Q2(R1) guidelines, parameters such as specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness must be evaluated [8].

Although several analytical methods have been reported for the estimation of Zidovudine and Lamivudine either individually or in combination, there is still a need for a simple, cost-effective, and reliable RP-HPLC method for routine quality control analysis [9,10].

Drug profile:

Zidovudine:

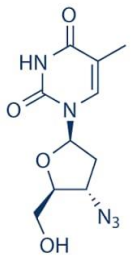

Fig.01 Structure of Zidovudine.

Nomenclature: 1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-5-methylpyrimidine-2,4(1H,3H)-dione

Molecular formula: C10H13N5O4

Molecular weight: 267.24 daltons

Solubility: In water

MOA: Zidovudine is phosphorylated intracellularly to zidovudine triphosphate, which inhibits HIV reverse transcriptase by competing with natural nucleotides and causes DNA chain termination.

Lamivudine:

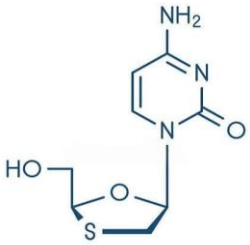

Fig.02 Structure of Lamivudine.

Nomenclature: (2R,cis)-4-amino-1-(2- hydroxymethyl- 1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

Molecular formula: C8H11N3O3S

Molecular weight: 229.3 Daltons

Solubility: Soluble in Water, sparingly soluble in Methanol

MOA: Lamivudine phosphorylates to lamivudine triphosphate, also inhibiting reverse transcriptase.

Sample solution preparation:

1. Accurately weigh and transfer equivalent to 1442 mg of Lamivudine and Zidovudine sample into a 100 ml volumetric flask.
2. Add about 70 ml of diluent And sonicate it to dissolve it completely and make up to the mark with the same Solvent.
3. Further pipette 0.1 ml of Lamivudine and Zidovudine of the above stock solution Into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Zidovudine–Lamivudine combination standard solution:

1. Weigh and transfer accurately 10mg of Lamivudine and 20 mg of Zidovudine working standard into a 10 ml volumetric flask.
2. Then add about 7 ml of Diluent and sonicate it to dissolve it completely and make up to the mark with the Same solvent.
3. Pipette out 0.3 ml of the above stock solution into a 10 ml volumetric flask and Dilute up to the mark with the diluent.

Preparation of mobile phase:

Mix a mixture of HPLC grade water 500 ml (50%), 500 ml of Methanol HPLC (50%) and degas it in ultrasonic water bath for 5 minutes.

Filter it through 0.45µ filter Under vacuum filtration.

RESULTS AND DISCUSSION

Lamivudine:

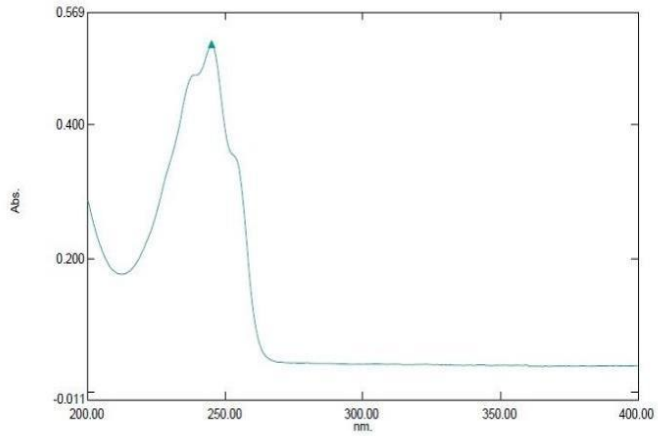

Fig.03 Determination of absorption maxima by UV/Visible Spectroscopy of Lamivudine.

Discussion: The resulting solution was scanned in the range of 200 nm to 400 nm. From spectrum 244 nm was selected as detection wavelength. At this wavelength Lamivudine showed good absorbance.

Zidovudine

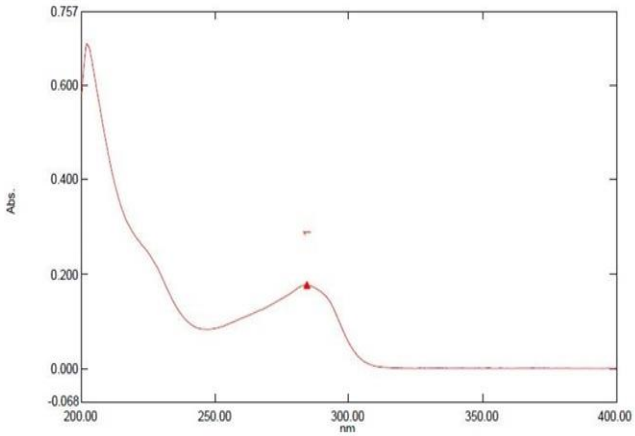

Fig. 04 Determination of absorption maxima by UV/Visible Spectroscopy of Zidovudine.

Discussion: The resulting solution was scanned in the range of 200 nm to 400 nm. From spectrum 284nm was selected as detection wavelength. At this wavelength Zidovudine showed good absorbance.

Method Development

Trial method 1:

Chromatographic Conditions:

Stationary phase: C18 column (100 x 4.6 mm i.d 5m)

Mobile phase: : Buffer pH4.6: methanol (50:50v/v)

Flow rate: 0.6 ml / min

Detector wavelength : 281 nm

Column temperature : Ambient

Injection volume : 20 µL

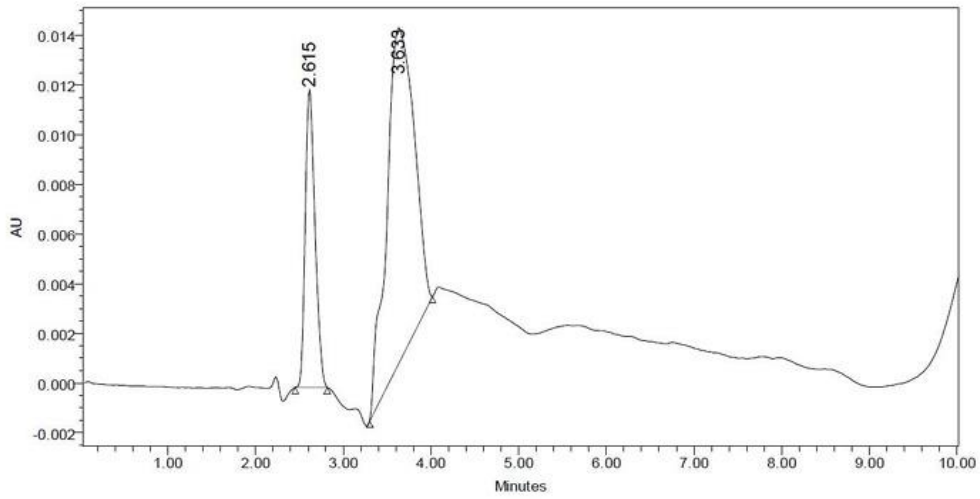

Fig .05 Chromatogram for trail 1.

Discussion: System suitability was failed. Fronting and tailing of the peaks was seen, retention time was more so another trail was performed.

Trial method 2:

Chromatographic Conditions:

Stationary phase: Waters C18 column (100 mm x 4.6 mm i.d, 5m)

Mobile phase: Methanol: Water (70:30v/v)

Flow rate: 0.6 ml/min

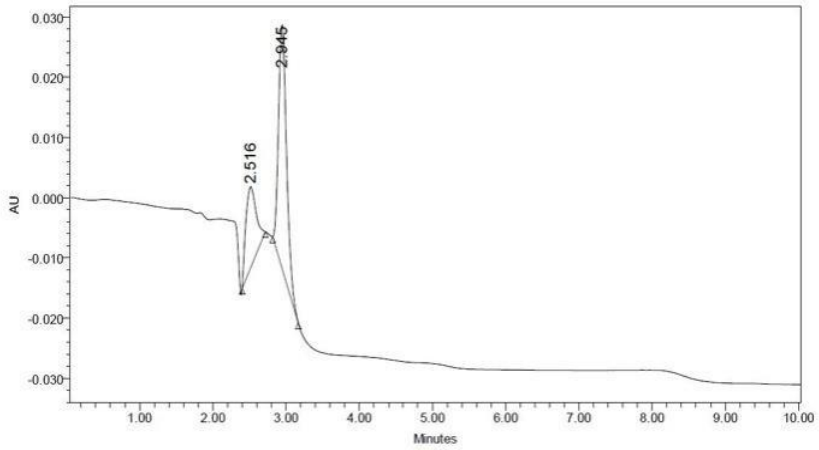

Fig .06 Chromatogram for trial 2.

Discussion: System suitability was failed. Resolution was not good.

Trial method 3:

Chromatographic Conditions:

Mobile phase: Methanol: Water (40:60v/v)

Stationary Phase:C18 (4.0×150 mm i.d, 5µm)

Flow rate: 0.6 ml/min

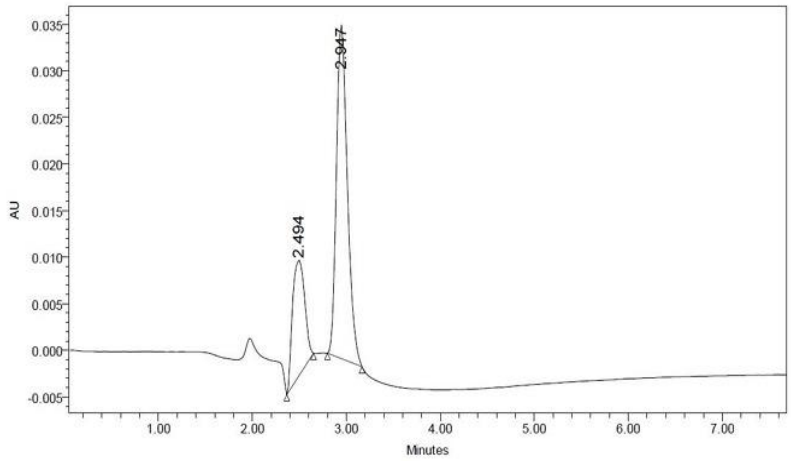

Fig. 07 Chromatogram for trial 3.

Discussion: System suitability was failed. Resolution was not good

Optimized method:

Chromatographic Conditions

Stationary phase: C18 column (150 x 4.6 mm i.d, 5 m)

Mobile phase: Methanol: Water (50:50v/v)

Flow rate: 0.6 ml/min

Detector wavelength: 281 nm

Column temperature: Ambient

Injection volume: 20 µL

Run time : 10 min

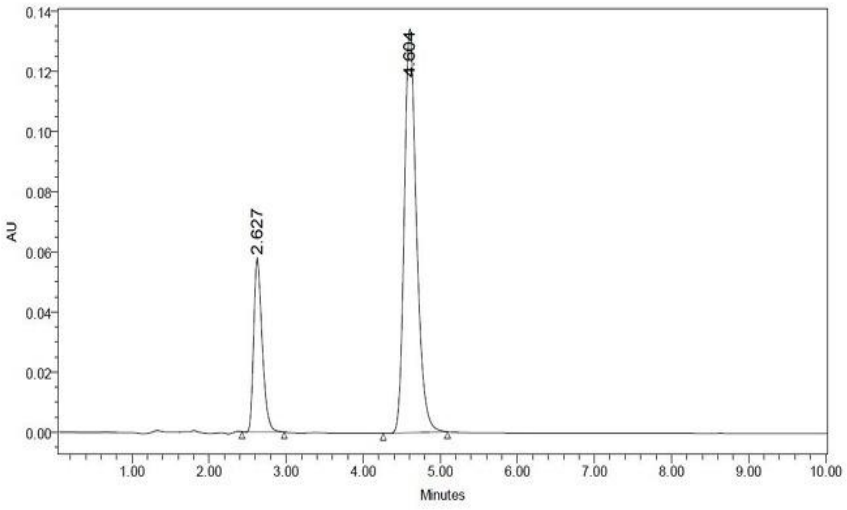

Fig .08 Chromatogram for optimized method.

Discussion: Resolution between two analytes is good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found the within the acceptance criteria. Accordingly, the method was regarded as optimized.

Analytical performance parameters

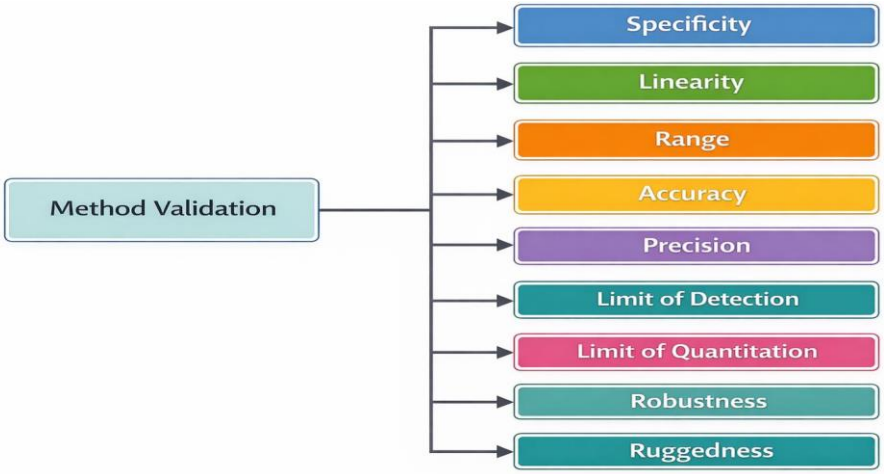

Fig: 09 The USP Nine Steps of Method Validation.

Method Validation Parameters and Results for Lamivudine and Zidovudine

Parameter	**Test / Description**	**Lamivudine**	**Zidovudine**	**Acceptance Criteria (ICH Q2R1)**
System Suitability	%RSD of 5 replicate injections, tailing factor, plate count	%RSD = 0.2%; Tailing = 1.4; Plates = 2843	%RSD = 0.2%; Tailing = 1.4; Plates = 2685	%RSD < 2%; Tailing < 2; Plates > 2000

Parameter	Test / Description	Lamivudine	Zidovudine	Acceptance Criteria (ICH Q2R1)
Specificity	At the analyte's retention time, no peaks arising from the blank or placebo were detected, indicating absence of interference.	No interference	No interference	No interfering peaks
Linearity	Concentration range (µg/mL) and correlation coefficient (r^2)	10–50 µg/mL (r^2 = 0.999)	20–100 µg/mL (r^2 = 0.999)	$r^2 \geq 0.999$
Precision (Repeatability)	5 replicate injections, same day	%RSD = 0.2	%RSD = 0.2	%RSD < 2%
Intermediate Precision (Ruggedness)	5 replicate injections, different day	%RSD = 0.3	%RSD = 0.2	%RSD < 2%
Accuracy (Recovery)	Recovery at 50%, 100%, 150% levels	Mean = 99.7%	Mean = 100.3%	98% – 102%
LOD	Based on signal-to-noise (S/N = 3:1)	0.7 µg/mL	0.3 µg/mL	S/N ≈ 3
LOQ	Based on signal-to-noise (S/N = 10:1)	2.7 µg/mL	1.0 µg/mL	S/N ≈ 10
Robustness	Variation in flow rate (±10%), wavelength (±5 nm), temperature (±5 °C)	No significant change	No significant change	%RSD < 2%, peak shape unaffected
Range	Interval showing accuracy, precision, and linearity	10–50 µg/mL	20–100 µg/mL	Should cover expected range
Assay (Content Uniformity)	Drug content in tablet formulation	99.9 % w/w	99.9 % w/w	98%–102%

CONCLUSION

The present study successfully developed and validated a simple, precise, and cost-effective RP-HPLC method for the simultaneous estimation of Lamivudine and Zidovudine in fixed-dose tablet formulations. The method complies with ICH Q2(R1) validation parameters, showing high accuracy, excellent precision, and specificity. Its short run time and minimal solvent use make it environmentally friendly and suitable for routine quality control in pharmaceutical industries. The reliability and reproducibility of the method suggest its potential application in stability studies and batch release analysis of antiretroviral formulations.

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