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Simultaneous Estimation of Stavudine and Zidovudine in Tablet Dosage Forms by a Validated RP-HPLC Method for Quality Control Applications

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ABSTRACT

Using a UV-Visible detector, a straightforward, repeatable, practical, and creative reversed-phase high performance fluid chromatographic technique was created and verified for the simultaneous measurement of stavudine and zidovudine in pharmaceutical measure forms. Acetonitrile: Water (50:50) was used as the mobile phase in the procedure, which was run on a C18 column at a run speed of 0.5 mL/minute. 283 nm was the ideal wavelength for detection, yielding a superior drug detector response. 15 min was the runtime. The method's linearity, accuracy, precision, specificity, toughness, stability, boundary of quantification (LOQ), and limit of detection (LOD) were all verified. The suggested approach was effectively used for the instantaneous measurement of zidovudine and stavudine in pharmaceutical dose forms.

Keywords: Stavudine, Zidovudine, RP-HPLC, Method development, Method validation.

INTRODUCTION

Antiretroviral treatment (ART) aims to achieve clinically meaningful immune reconstitution, which includes increases in naïve CD4+ cells and the reappearance of HIV- and other pathogen-specific lympho proliferative responses. The goal of the current investigation was to evaluate how zidovudine and stavudine sequencing affected the responses of CD4 lymphocytes and plasma HIV-1 RNA in individuals in the CHORUS observational cohort. Numerous individual analytical techniques are available for estimating the medications listed above in their separate dose forms or in combination with other dosage forms, according to the literature study[1-2]. One dideoxynucleoside that is used to treat HIV infection is stavudine. The nucleoside overturn transcriptase inhibitor (NRTI) stavudine has capabilities. Phosphorylation of stavudine results in active metabolites that vie for viral DNA inclusion. They function as a sequence terminator of DNA combination and competitively block the HIV reverse transcriptase enzyme. Viral DNA development is stopped because the integrated nucleoside analogue lacks a 3'-OH group, which

inhibits the formation of the 5' to 3' phosphodiester linkage necessary for DNA chain elongation[3-4].

Zidovudine, often known as azidothymidine or AZT, is an analog of thymidine. 1-[(2R,4S,5S)-4-azido-5-(hydroxyl methyl) oxolan-2yl] is its chemical name. Tetrahydropyrimidine-2,4-dione -5methyl-1,2,3,4-. It works by specifically blocking HIV's overturn transcriptase, an enzyme the virus utilizes to replicate its RNA into DNA. The synthesis of HIV's double-strands DNA, which is then incorporated into the inherited substance of the tainted cell, requires reverse transcription. AZT has a roughly 100-fold higher affinity for HIV's overturn transcriptase, but at extremely high dosages, its triphosphate form may also block DNA polymerase, which is necessary for human cells to divide. It has been shown that the selectivity results from the cell's capacity to swiftly fix its have DNA sequence in the event that AZT breaks it during synthesis, something that the HIV virus is unable to do. As a result, AZT prevents HIV replication while leaving uninfected cells functioning normally[5-6].

This review's objectives are to provide an overview of the field's history, explain the techniques used to find agents, and highlight potential antiviral medications that are already on the market and in development. Antivirals are especially used to treat viruses and highlight new and modern approaches to medication production as well as to stop the spread of viruses that are resistant to treatments.

EXPERIMENTAL

Chemicals and Reagents

Intas Pharmaceuticals Pvt. Ltd. provided the commercially available formulations of stavudine and zidovudine (API) as well as other necessary compounds for the study. We bought additional reagents from Rankem Ltd., including methanol, acetonitrile, HPLC water, buffer, orthophosphoric acid, triethylamine, 1N HCL, 1N NaOH, and 3% H_2O_2. Additionally, every reagent was of analytical quality[7].

Optimized Chromatographic state

HPLC testing circumstances were improved using the Cosmosil C18 analytical column, which is 250 mm long, 4.6 mm wide, and has a particle size of 5µm.

Preparation of Solutions

Water containing orthophosphoric acid (0.1% V/V): Pipette 0.6 mL of orthophosphoric acid into a 500 mL measuring cylinder filled with 250 mL of HPLC-grade water, then top it over with the remaining 500 mL of HPLC mark water. Moved into a container of reagents and properly assorted the contents and kept at room heat. Three days after the preparation date, this solution was put to use[8].

Mobile phase: Acetonitrile: 0.1% OPA (54:44 % V/V)

450 mL of 0.1% OPA and 550 mL of aceto nitrile were taken out of a measuring cylinder, put into a reagent container, and properly assorted. Stored at room temperature after the preparation date, this solution was utilized within three days. This served as the diluent.

Stavudine Stock Solution, 4000 µg/ mL

40 mg of standard stavudine, precisely weighed, was put into a 10 mL vol. flagon, and the right quantity of methanol was further to get the ultimate stavudine concentration of 4000 µg/mL. The solution was utilized within seven days after the manufacture date and kept in a refrigerator at 5±3°C[9].

Zidovudine Stock Solution, 800 µg/ mL

8 mg of accurately measured standard Zidovudine was placed into a 10 mL vol. flask, and the suitable vol. of methanol was further to achieve the target closing attention of 900 µg/mL. The solution was utilized within seven days after the manufacture date and kept in a refrigerator at 5±3°C[10].

Combine Stock solution, (Stavudine 40 µg/mL and Zidovudine 9 µg/mL)

0.1 milliliters of hoard in a 10.0 mL volumetric flask, a solution of Stavudine 4000 µg/mL and Zidovudine 800 µg/mL was transferred. To get a solution with concentrations of Stavudine 40 µg/mL and Zidovudine 8 µg/mL, the volume was adjusted to the proper level using diluents. The solution was utilized within seven days after the preparation date and kept in a refrigerator at 5±3°C.

Method Validation

Linearity

By diluting the standard stock solution to provide a final concentration in the variety of

5–25 g/ml for Lamivudine and 10–50 gram/ml for Zidovudine for 5 g/mL injection, linearity was achieved, and a calibration curve was created.

Precision (Repeatability)

In order to study the method's intraday accuracy, the drug combination was repeatedly injected on the equal date. Following five measurements, The coefficient of variation was determined to be 5–25 µg/mL for stavudine and 10–50 µg/mL for zidovudine[11].

Precision (Intermediate)

Three duplicates of the standard concentration were injected by several analyzers to achieve intermediate accuracy. The percentage RSD was computed.

Accuracy

Three distinct concentrations of sample solutions were made: 80%, 100%, and 120%. A known quantity of sample was added to each solution, and the recovery of the additional sample was examined[12].

Robustness

The robustness of the RPHPLC method for the concurrent assessment of Stavudine and Zidovudine, along with their %RSD, was assessed by examining the effects of minor alterations in chromatographic conditions, including a pour rate variation of ±0.1 mL/min, a detection wavelength variation of ±1 nm, and a mobile phase variation of ±1 mL[13].

Limit of quantification (LOQ)

Limit of quantification is the lowly analyte deliberation that can be identified under the specified experimental circumstances with a reasonable level of precision and accuracy[14].

RESULT AND DISCUSSION

Assortment of immobile phase

Together Stavudine and Zidovudine are polar compounds, so analysed by means of a C18 column.

Assortment of wavelength

Since the wavelength chosen affects the HPLC method's selectivity, 265 nm was chosen as the detection wavelength for Stavudine and Zidovudine in order to ensure that both medications responded well.

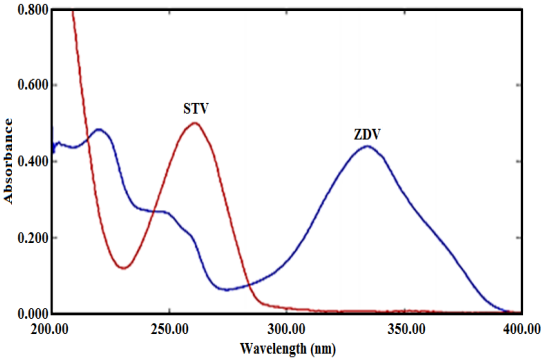

Fig. 1. Wavelength selection was based on the overlaid UV spectra of STV (20 µg/mL) and ZDV (4.5 µg/mL)

Establishment of the retention time of individual drugs

To check for separation, a thinned assorted standard of the medicines was administered into the arrangement. The following parameters were ultimately determined to provide an optimum chromatogram after slight adjustments were made to the flow rate in order to increase resolution and the number of theoretical plates.

- HPLC manufacturer: Agilent Laboratories 1100 slope arrangement
- Particle size of stuffing: 5µm
- The immobile stages: C18COSMOSIL (250mm x 4.6mm, 5 microns)
- Detection wavelength: 283 nm
- Flow rate: 0.7 mL/min
- Movable Phase: Acetonitrile and 0.1% orthophosphoric acid (OPA) in a 54:44 %v/v ratio Temperature: Ambient
- Injection Volume: 20 µL

The retention times and theoretical plate counts for both drugs are presented in Table 1, and the corresponding chromatogram is shown in Figure 2.

Table 1: Retention time of STV and ZDV

Parameter	Stavudine	Zidovudine
Elapsed Retention Time (min)	3.668	5.925
Theoretical plates	5534	17824
Tailing factor	0.924	0.80
Declaration	-	10.8

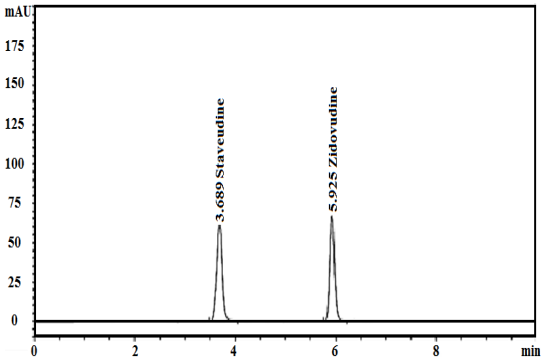

Fig. 2. Chromatogram for disjointing of Stavudine with Zidovudine

System suitability

For the conventional solutions, the tailing factor, resolution, and column efficiency were computed. The findings indicated that the approach was suitable for analysing the selected drug combinations. When the method is consistently executed, the system's suitable parameters may reside inside a 2% relative variance range. Fig. 3 shows the chromatogram, and Table 2 shows the system suitability parameter comparison findings.

Table 2: Proportional consequences of together the drugs

Parameter	Stavudine	Zidovudine
Elapsed Retention Time (min)	3.75	5.99
Separation stage	5523.33	17823.17
Peak tailing ratio	0.91	0.80
Resolution	10.8	

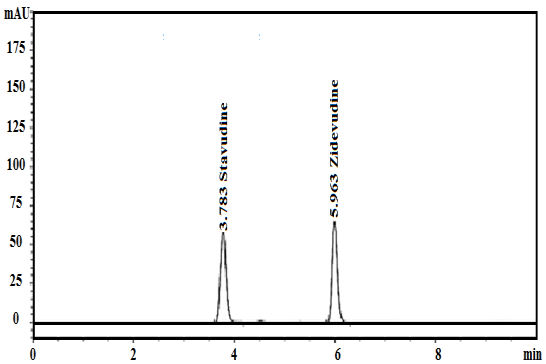

Fig. 3. All scheme appropriateness variables of the developed process comply through its customary

Method corroboration

Specificity

By injecting solutions of both medications, the method's selectivity was examined. Sofosbuvir and Ledipasvir were found to have two distinct peaks at retention durations of 3.776 and 3.763, 6.004 and 5.993 minutes for the customary and illustration, correspondingly. The approach was specific since the drug standards plus the pharmaceuticals from model solutions had the identical retention periods Figure 4.

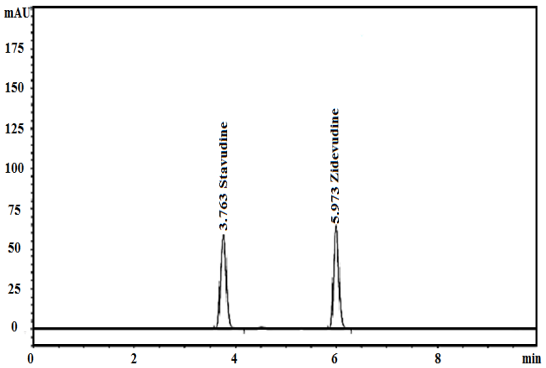

Fig. 4. Chromatogram of Stavudine and Zidovudine Sample

Linearity

Five concentration levels were used to assess the method's linearity. The response factor was graphed vs the drug concentration to establish the calibration curve. Stavudine and Zidovudine exhibit linearity within the attentiveness ranges of 39–199 µg/mL and 8–44 µg/mL, correspondingly. To get the calibration curve, construct a graph of Area against Concentration. Table 3 and the calibration curve in Fig. 5 illustrate the linearity of Stavudine, whereas Table 4 and the calibration curvature in Fig. 6 demonstrate the linearity of Zidovudine. The calibration curvature was establish to be linear.

Table 3: Linearity statistics of Stavudine

Conc. (µg/mL)	Peak Area 1	Peak Area 2	Regular Peak area (n=2)
0	0	0	0
40	238.994	237.907	238.45
80	468.212	471.527	469.87
120	715.665	713.112	714.389
160	942.344	937.266	939.805
200	1200.008	1205.24	1202.624

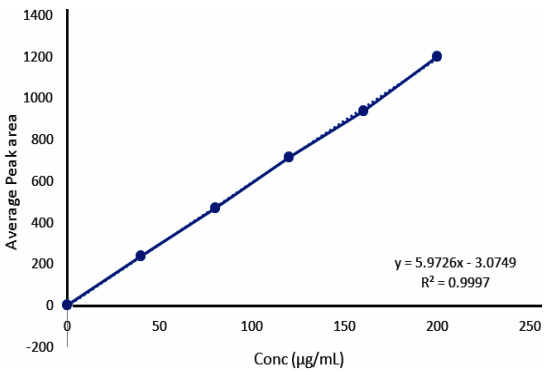

Fig. 5. Calibration plot for Stavudine

Table 4: Linearity statistics of Zidovudine

Conc (µg/mL)	Peak Area 1	Peak Area 2	Normal Peak area (n=2)
0	0	0	0
9	216.571	215.947	216.259
18	444.776	446.687	445.731
27	665.105	667.325	666.215
36	899.079	897.853	898.466
45	1139.157	1129.372	1134.264

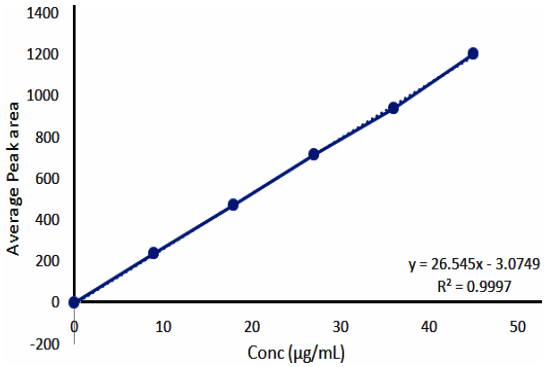

Fig. 6. Calibration plot for Zidovudine

Precision

The method's accuracy was shown by research on structure repeatability (intraday precision) and transitional precision (inter-day precision) variations.

Scheme Repeatability (Intra-Day precision)

Six duplicates of the produced sample solutions were used to assess the repeatability of the system. For intra-day precision, the assay was performed six period at concentration level of 40,80, and 120 µg/ mL for Stavudine and 9, 8, and 27 µg /mL for Zidovudine. This allowed for the determination of climax area for the medications and the repeatability of sample application. RSD was computed and found to be less than 2% when peak locations were identified.

Transitional Precision (InterDay precision)

Three duplicates of the produced sample solutions were used to calculate the intermediate precision. The evaluation of three example sets conducted on distinct days at concentration level of 80 µg/mL for Sofosbuvir and 18 µg/mL for Ledipasvir over various instance intervals to assess interday accuracy yielded the intermediate accuracy of sample application and peak area measurement. RSD was computed and found to be less than 2% when peak locations were identified.

Accuracy

By using the conventional addition procedure, which involves analyzing formulation samples to which specific quantities of genuine pharmaceuticals were added, the techniques' correctness was guaranteed. The final mixes were tested, and the outcomes for both medications were contrasted with the predictions. Three separate quantities of standards (Stavudine 31,39,47 µg/mL and Zidovudine 7.1,9,10.7µg/mL) were spiked into beforehand analyze sample of the tablets (Stavudine 39 µg/mL and Zidovudine 8 µg/mL) in order to conduct the recovery tests in triplicate. The recovery investigations were conducted in compliance with ICH Guidelines, and the excellent recoveries using the standard addition technique demonstrate the good accuracy.

Robustness

Deliberate changes were made to the procedure parameters, including flow rate, pH, and wavelength, in order to assess the robustness of the suggested approach. It was found that the method's analytical performance did not significantly alter. According to the findings, the technique's robustness was shown by the short values of %RSD (<2) of % drug substance that were achieved after making minor adjustments to the method parameters.

Limit of Detection and Limit of Quantification

The ICH Guidelines were adhered to in establishing the limits of detection (LOD) and quantitation (LOQ) for both stavudine as well as zidovudine. The limit of detection (LOD) was considered as 3.3/S and the limit of quantification (LOQ) as 10/S, base on the typical divergence of the answer and grade of the calibration curvature established in a short concentration range of the objective analyte. S represents the slope of the calibration curve, whereas (the standard divergence of the rejoinder) was unwavering using the standard divergence of the y-intercepts of the deterioration lines.

Table 5: Limit of Detection (LOD) and Limit of Quantification (LOQ)

Sr. No	DRUG	LOD (µg/mL)	LOQ (µg/mL)
1	Stavudine	0.420	1.27
2	Zidovudine	0.051	0.177

Assay

Established HPLC technique was used for the examination of a commercial tablet formulation.

Table 6: %Results of Assay of marketed formulation

Marketed Formulation	Ingredients	Conc. µg/mL	Area (n=3)	Amount Found (µg/mL)	% Assay
Harvoni	Stavudine	80	460.86	78.36	98.20%
	Zidovudine	18	452.285	17.284	100.58%

CONCLUSION

Goal of the current effort is to develop the RP-HPLC technique and optimize the chromatographic conditions. The suggested approach may be effectively used for the assessment of stavudine and zidovudine in pharmaceutical dose forms since it is quick, easy, sensitive, accurate, robust, and exact. The percentage RSD from five duplicate injections for Zidovudine and Stavudine retention durations and peak areas was used to assess the system appropriateness characteristics using standard chromatograms. Lamivudine and zidovudine were found to have linearity and correlation coefficients of 0.999. It was done with precision.

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Conflict of interest

The author declare that we have no conflict of interest.

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