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STABILITY INDICATING HPLC METHOD FOR THE ESTIMATION OF LAMIVUDINE AND STAVUDINE IN COMBINED DOSAGE FORM

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ABSTRACT

This study presents the development and validation of a stability-indicating HPLC method for the simultaneous estimation of Lamivudine (LAMI) and Stavudine (STAV) in combined dosage forms. The method demonstrated excellent linearity with correlation coefficients of 0.9990 for LAMI and 0.999 for STAV across specified concentration ranges. Recovery studies indicated high accuracy, with mean recoveries of 98.08% to 98.91% for LAMI and 98.48% to 99.03% for STAV at different recovery levels. The method exhibited high precision, as evidenced by low %R.S.D. values across various validation parameters. Sensitivity was confirmed through low LOD (0.12 µg/ml for LAMI; 0.10 µg/ml for STAV) and LOQ (0.40 µg/ml for LAMI; 0.25 µg/ml for STAV). Forced degradation studies showed that both drugs maintained stability under standard conditions, although they were susceptible to degradation under acidic and alkaline environments. The validated HPLC method is robust and suitable for routine quality control of LAMI and STAV in pharmaceutical formulations, ensuring the reliability of therapeutic products.

Keywords: Lamivudine, Stavudine, HPLC, Stability-indicating method, Validation, Recovery studies, Forced degradation, Pharmaceutical formulations, Quality control.

INTRODUCTION

Lamivudine and Stavudine are two nucleoside reverse transcriptase inhibitors (NRTIs) widely used in the management of HIV/AIDS. Both drugs are effective in inhibiting viral replication, leading to improved clinical outcomes in patients. Lamivudine (3TC), chemically known as 4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)(2R, 4S)-2-(3-thiazolyl)butan-1-ol, and Stavudine (d4T), also known as 2',3'-didehydro-2',3'-dideoxythymidine, play crucial roles in antiretroviral therapy (ART) by targeting the reverse transcriptase enzyme, thereby blocking the conversion of viral RNA into DNA (Gupta *et al.,* 2020; WHO, 2016).

The combined dosage form of Lamivudine and Stavudine offers the advantage of simplifying the treatment regimen, thereby improving patient adherence to therapy (Bukhari *et al.,* 2018). However, the stability of these compounds in formulations is a significant concern, as factors such as temperature, humidity, and light can adversely affect their efficacy. Degradation of these active pharmaceutical ingredients (APIs) can lead to reduced therapeutic effectiveness or the formation of potentially harmful degradation products (Snyder *et al.,* 2019).

To ensure the safety and efficacy of combined dosage forms, robust analytical methods are essential for the estimation of Lamivudine and

Stavudine. High-Performance Liquid Chromatography (HPLC) is a widely accepted technique due to its sensitivity, precision, and ability to separate compounds in complex mixtures (Zhou *et al.,* 2021). A stability-indicating HPLC method is crucial, as it can differentiate between the active drugs and their degradation products, providing reliable data on the stability of the formulation (ICH, 2003).

In this study, we aim to develop and validate a stability-indicating HPLC method for the simultaneous estimation of Lamivudine and Stavudine in combined dosage forms. This method will facilitate the assessment of drug stability under various conditions and support regulatory compliance, ensuring the quality of the pharmaceutical product.

MATERIALS AND METHODS

Selection of mobile phase

Initially to estimate Lamivudine and Stavudine in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10 mg API of LAMI and STAV was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of LAMI and STAV respectively (Stock-B).

Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml, for LAMI. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of STAV also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml for LAMI and 1-5µg/ml for STAV were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 280.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived (Kathiresan *et al.,* 2009).

System suitability parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of LAMI 10µg/ml for LAMI and 5µg/ml STAV was injected separately. Peak report and column performance report were recorded for all chromatogram.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components (Kapil *et al.,* 2011).

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Khan *et al.,* 2008).

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for LAMI and 1, 2, 3, 4 and 5µg/ml for STAV indicates the precision under the same operating condition over short interval time (Sudhakar *et al.,* 2010).

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations (Shah *et al.,* 2012).

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected (Dhoka *et al.,* 2010). The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of LAMI and 6mg of STAV was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL LAMI and 6µg/mL STAV respectively. The amounts of LAMI and STAV in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected [41].

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC

and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

50 mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

RESULTS AND DISCUSSION

The results presented for the linearity of Lamivudine (LAMI) and Stavudine (STAV) demonstrate excellent correlation coefficients, indicating strong linear relationships between concentration and absorbance. For LAMI, the linear range was established between 5-25 µg/ml with a correlation coefficient (r^2) of 0.9990, and a slope of 177.96. Similarly, STAV showed a linear range of 1-5 µg/ml with an r^2 of 0.999 and a slope of 311.3. These results confirm that the developed HPLC method is suitable for quantifying both drugs in their respective ranges.

The recovery studies performed on marketed formulations yielded commendable results, suggesting that the HPLC method is accurate and reliable for the quantification of both LAMI and STAV. At recovery levels of 80%, 100%, and 120%, the mean recovery percentages for LAMI were 98.08%, 98.36%, and 98.91%, respectively, while for STAV, the recoveries were 99.03%, 98.48%, and 98.89%. The low standard deviations further support the consistency of the method.

The validation results demonstrate the method's precision, with percent relative standard deviations (%R.S.D.) for repeatability, day-to-day variations, reproducibility, analyst-to-analyst comparisons, and robustness all within acceptable limits. For LAMI, the repeatability was 99.361 ± 0.054, and for STAV, it was 98.245 ± 0.044, indicating high reliability in quantifying these compounds.

Limit of Detection (LOD) and Limit of Quantification (LOQ) values were found to be quite low for both LAMI (LOD: 0.12 µg/ml, LOQ: 0.40 µg/ml) and STAV (LOD: 0.10 µg/ml, LOQ: 0.25 µg/ml). These results highlight the sensitivity of the HPLC method, making it suitable for detecting and quantifying low concentrations of these drugs in pharmaceutical formulations.

In terms of assay results, the findings indicate that the assay method effectively measures the labeled amounts of LAMI and STAV in tablet formulations. The percentage found for LAMI was 148.85 mg, and for STAV, it was 59.85 mg, leading to % assays of 99.23% and 99.75%, respectively. The low %RSD values (0.125 for LAMI and 0.226 for STAV)

suggest that the method is reproducible and reliable.

Forced degradation studies are critical for understanding the stability of pharmaceutical compounds under various stress conditions. For LAMI, the standard drug recovered 99.78%, with significant degradation observed during acidic hydrolysis (13.13% decomposition) and alkaline hydrolysis (19.55% decomposition). Oxidative and photolytic conditions showed moderate stability with 6.46% and 8.04% decomposition, respectively.

Similarly, STAV demonstrated 99.95% recovery under standard conditions, but was more susceptible to degradation under acidic and alkaline conditions, with 16.63% and 18.81% decomposition, respectively. The oxidative degradation resulted in 8.8% decomposition, and photolytic degradation showed 6.63%. These results indicate that both LAMI and STAV are relatively stable under standard conditions but may require protective measures during storage and handling to minimize degradation.

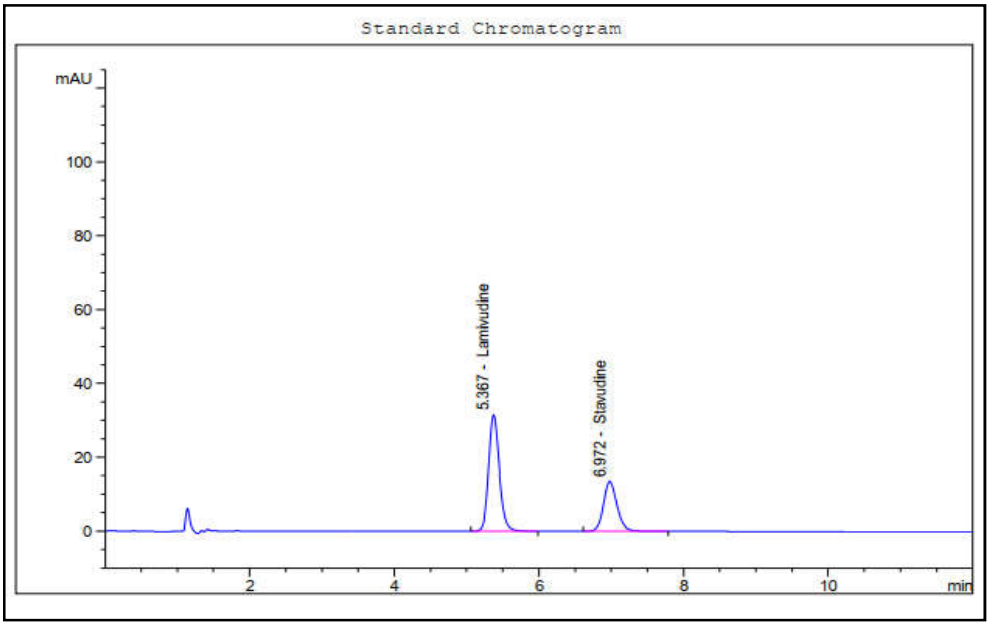

Figure 1: Chromatogram of Both the drug

Table 1: Results of Linearity of Lamivudine (LAMI) and Stavudine (STAV)

	Results of Linearity	**Stavudine**
Parameter	**LAMI**	**STAV**
Range (µg/ml)	5-25(µg/ml)	1-5(µg/ml)
Correlation Coefficient (r^2)*	0.9990	0.999
Slope (m)*	177.96	311.3
Intercept (c)*	3.4298	3.2107

*Average of five determination

Table 2: Results of recovery studies on marketed formulations

Recovery Level %	% Recovery (Mean±SD)*	
	LAMI	STAV
80	98.08±1.158	99.03±0.241
100	98.36±0.899	98.48±0.421
120	98.91±0.772	98.89±0.962

Table 3: Results of validation

		Parameter (Mean±SD)*	
		LAMI	STAV
Precision (%R.S.D.)*	Repeatability	99.361±0.054	98.245±0.044
	Day to Day	99.055±0.065	97.347±0.023
	Reproducibility	99.527±0.020	98.165±0.020
	Analyst to Analyst	98.964±0.171	98.165±0.030
	Robustness	99.232±0.063	97.542±0.056

*Average of five determination

Table 4: LOD and LOQ of LAMI and STAV

Name	LOD (µg/ml)	LOQ (µg/ml)
LAMI	0.12	0.40
STAV	0.10	0.25

Table 5: Result of assay of tablet formulation

	LAMI*	STAV*
Label Claim (mg)	**150mg**	**60mg**
% Found (mg)	148.85	59.85
% Assay	99.23	99.75
% RSD	0.125	0.226

*Average of three determination

Table 6: Results of Forced degradation studies of LAMI

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.78	0
Acidic hydrolysis	86.65	13.13
Alkaline hydrolysis	80.23	19.55
Oxidative degradation	93.32	6.46
Photolytic degradation	91.74	8.04

Table 7: Results of Forced degradation studies of STAV

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.95	0
Acidic hydrolysis	83.32	16.63
Alkaline hydrolysis	81.14	18.81
Oxidative degradation	91.15	8.8
Photolytic degradation	93.32	6.63

CONCLUSION

The developed stability-indicating HPLC method is validated and suitable for the simultaneous estimation of Lamivudine and Stavudine in combined dosage forms. The results affirm the method's accuracy, precision, sensitivity, and reliability, making it a valuable tool for quality control in pharmaceutical manufacturing and regulatory assessments. Further studies could explore the long-term stability of these compounds and their formulations under various environmental conditions.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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