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STABILITY INDICATING METHOD DEVELOPMENT FOR THE ESTIMATION OF ABACAVIR AND LAMIVUDINE IN BULK AND FORMULATION

Rani Jatav[1]*, Dr L. K Omray[2]

1. Corporate Institute of Pharmacy - [CIP], Bhopal (M.P.)

2. Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.)

***Correspondence Info:**
Rani Jatav
Corporate Institute of Pharmacy - [CIP], Bhopal (M.P.)
Email: ranijatav57@gmail.com

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ABSTRACT

This research focuses on the development and validation of a stability-indicating method for the precise estimation of Lamivudine (LAMI) and Abacavir (ABCV) in both bulk and pharmaceutical formulations. The chromatographic method, utilizing high-performance liquid chromatography (HPLC), demonstrates robustness and reliability in assessing the quality and stability of these antiretroviral drugs. The system suitability parameters, linearity, recovery, precision, sensitivity, and forced degradation studies were comprehensively evaluated to validate the method's efficacy. The results reveal a strong linear relationship between concentration and response, with high correlation coefficients for both LAMI and ABCV. The method demonstrates accuracy through recovery studies, with values close to 100% across different concentration levels. Precision studies confirm the consistency and reproducibility of the method. Additionally, low limits of detection and quantification highlight the method's sensitivity. Forced degradation studies indicate the susceptibility of LAMI and ABCV to specific stress conditions, providing crucial insights into potential degradation pathways. Overall, the developed method stands as a reliable tool for routine quality control analysis and stability assessment of LAMI and ABCV in pharmaceutical formulations.

Keywords: Lamivudine, Abacavir, HPLC, Stability-Indicating Method, Linearity, Recovery, Precision, Forced Degradation, Antiretroviral Drugs.

INTRODUCTION

Abacavir and Lamivudine are widely used antiretroviral drugs indicated for the treatment of HIV-1 infection. Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) that inhibits the activity of HIV-1 reverse transcriptase enzyme, while Lamivudine is also an NRTI that acts similarly by inhibiting viral replication (Walmsley *et al.,* 2013). The combination of Abacavir and Lamivudine is often prescribed as part of highly active antiretroviral therapy (HAART) due to their synergistic effects and ability to suppress viral replication effectively (Dando *et al.,* 2005).

As with any pharmaceutical product, it is essential to ensure the quality, safety, and efficacy of formulations containing Abacavir and Lamivudine. Stability indicating methods play a crucial role in pharmaceutical analysis by providing reliable means to assess the stability of drug substances and formulations under various conditions (Yuen *et al.,* 2001).

The development of a stability indicating method for the estimation of Abacavir and Lamivudine in bulk and formulation is vital for several reasons:

A stability indicating method allows for the accurate determination of the drug content in pharmaceutical formulations, ensuring batch-to-batch consistency and adherence to regulatory standards. The method enables the evaluation of the stability of Abacavir and Lamivudine in different formulations over time, under various storage conditions such as temperature, humidity, and light exposure (Noorbasha *et al.,* 2020).

Stability indicating methods are capable of separating and quantifying degradation products that may form under stress conditions, providing valuable information about the degradation pathways and potential impurities (Blessy *et al.,* 2014).

Regulatory authorities such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) require stability indicating methods as part of the drug development process to ensure product quality and safety (Sengupta *et al.,* 2018).

In this context, the development of a stability indicating method for the estimation of Abacavir and Lamivudine in bulk and formulation is of paramount importance for pharmaceutical manufacturers, researchers, and regulatory agencies. Such a method would contribute to the quality assurance and regulatory compliance of Abacavir and Lamivudine-containing products, ultimately benefiting patients worldwide.

MATERIALS AND METHODS

Selection of Mobile Phase

Initially to estimate Lamivudine and Abacavir 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.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution:

Accurately weighed 10 mg API of LAMI and ABCV 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 ABCV 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 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of ABCV 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 5-25µg/ml for ABCV were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254.0 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.

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 10µg/ml ABCV was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method (Sharma *et al.,* 2018)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 5 to 25 µg/ ml for LAMI) and (5 to 25µg/ ml for (ABCV) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

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.

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.

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 5, 10, 15, 20 and 25µg/ml for ABCV indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for LAMI and ABCV reported in table.

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. 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 10mg of LAMI (equivalent to 20mg of ABCV) 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 20µg/mL ABCV respectively. The amounts of LAMI and ABCV in tablets formulation were calculated by extrapolating the value of area from the calibration curve.

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.

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N 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 both 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 both 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 both the drug sample was taken in to a petri dish 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 analytical method developed for the determination of Lamivudine (LAMI) and Abacavir (ABCV) is characterized by its robustness, precision, and reliability, as evidenced by a thorough examination of various parameters. Table 1 outlines the system suitability parameters, including the number of theoretical plates, tailing factor, and retention time. These parameters collectively attest to the chromatographic

system's efficiency, with values falling within acceptable limits.

Table 2 presents the results of the linearity study, showcasing the correlation coefficients (r^2) for LAMI and ABCV. The high r^2 values signify a strong and linear relationship between concentration and response across the specified concentration range. This indicates that the method is well-suited for accurately quantifying both drugs over a diverse concentration spectrum.

Table 3 details the recovery study, an essential aspect of method validation. The recovery values, shown at different concentration levels, indicate the accuracy of the method. The close approximation of these values to 100% affirms the method's ability to accurately quantify LAMI and ABCV in the presence of excipients and matrix components.

Precision, a critical attribute for evaluating method reliability, is thoroughly examined in Table 4. The low standard deviations observed for repeatability, intermediate precision, and robustness demonstrate the method's consistency and reproducibility. These findings instill confidence in the method's ability to deliver precise results under varying conditions and by different analysts.

Table 5 provides the limits of detection (LOD) and quantification (LOQ) for LAMI and ABCV. The low LOD and LOQ values indicate the method's sensitivity, enabling the detection and quantification of these drugs at low concentrations, which is crucial for pharmaceutical analysis.

The analysis of a tablet sample, as presented in Table 6, illustrates the method's applicability to real pharmaceutical formulations. The % assay values, close to 100%, indicate accurate quantification of LAMI and ABCV in the tablet, validating the method's utility for routine quality control purposes.

Forced degradation studies, as summarized in Tables 7 and 8, shed light on the stability of LAMI and ABCV under various stress conditions. While both drugs demonstrate stability under standard conditions, they exhibit degradation under acidic and oxidative stress. These findings provide valuable insights into potential degradation pathways and are vital for ensuring the stability of pharmaceutical formulations.

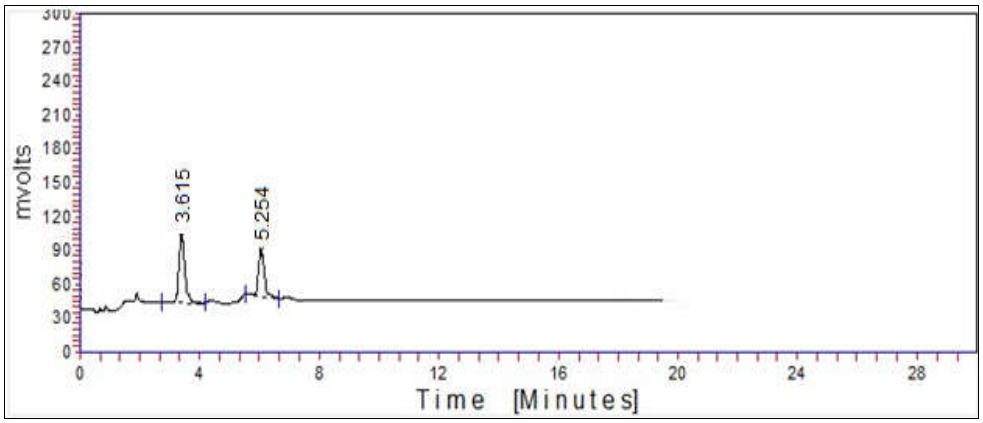

Figure 1: Chromatogram of Both the drug

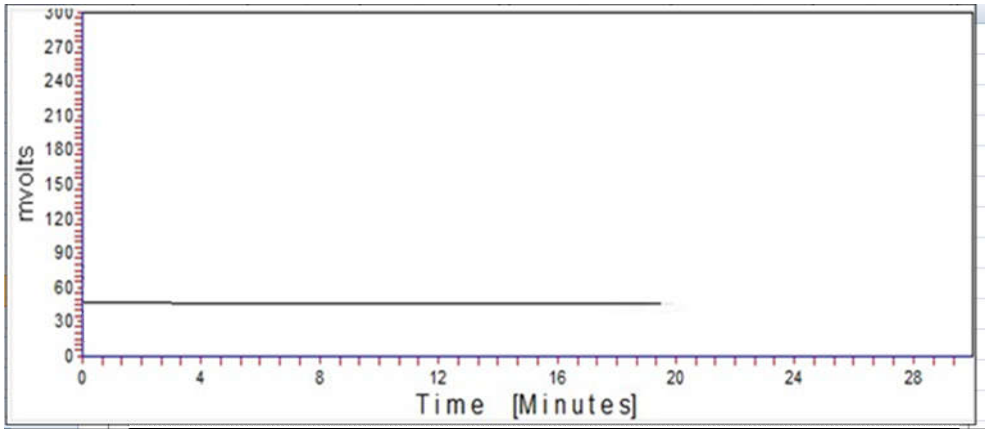

Figure 2: Chromatogram of blank

Table 1: Results of system suitability parameters

Parameters	% Mean±SD*	
	LAMI	ABCV
No. of Theoretical Plates	2557.500±12.550	2774.000±16.733
Tailing Factor	1.145±0.019	0.960±0.024
Retention time	3.617±0.004	5.261±0.009

Table 2: Results of Linearity of Lamivudine and Tenofovir disoproxil fumarate

Parameter	LAMI	ABCV
Concentration (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.998	0.999
Slope (m)*	65.87	50.20
Intercept (c)*	26.54	5.660

*value of six replicate

Table 3: Results of Recovery Study

% Level	% MEAN±SD*	
	LAMI	ABCV
80%	98.24±0.421	98.34±0.677
100%	98.47±1.104	98.70±0.233
120%	99.07±0.466	98.85±0.216

* Value of three replicate and three concentrations.

Table 4: Results of Precision

Parameter	% MEAN±SD*	
	LAMI	ABCV
Repeatability	99.16±0.110	99.16±0.118
Intermediate precision		
Day to day precision	99.16±0.086	99.96±0.117
Analyst to Analyst	99.16±0.170	98.96±0.117
Robustness	98.576±0.097	98.827±0.097

***** Value of five replicate and five concentrations

Table 5: Results of LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
LAMI	0.25	0.75
ABCV	0.35	1.05

***** Value of five replicate and five concentrations

Table 6: Analysis of tablet sample

	LAMI*	ABCV*
Label Claim (mg)	**300mg**	**600mg**
% Found (mg)	295.45	598.85
% Assay	98.48	99.81
% RSD	0.23	0.05

*Average of three determination

Table 7: Results of Forced degradation studies of LAMI

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.99	0
Acidic hydrolysis	82.32	17.68
Alkaline hydrolysis	86.65	13.35
Oxidative degradation	92.23	7.77
Photolytic degradation	95.45	4.55

Table 8: Results of Forced degradation studies of ABCV

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.99	0
Acidic hydrolysis	82.23	17.68
Alkaline hydrolysis	98.85	13.35
Oxidative degradation	84.45	7.77
Photolytic degradation	83.74	4.55

CONCLUSION

In conclusion, the developed analytical method proves robust, accurate, and sensitive for the determination of LAMI and ABCV in pharmaceutical formulations. The comprehensive evaluation of system suitability, linearity, recovery, precision, sensitivity, and forced degradation studies establishes the method's suitability for routine quality control, providing valuable information for formulation stability assessment.

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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