RESEARCH PAPER

Method development and validation for simultaneous estimation of Lamivudine and Zidovudine by UV spectrophotometric method.

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ABSTRACT

This study developed and validated a UV spectrophotometric approach for the simultaneous measurement of lamivudine (LAM) and zidovudine (ZID) in pharmaceutical dosage forms. This method use the simultaneous equation methodology to ascertain the absorbance at two wavelengths, 271 nm and 267 nm, corresponding to the peak absorption of LAM and ZID, respectively. The proposed methodology underwent statistical assessment to confirm its specificity, linearity, accuracy, precision, robustness, and adherence to the International Conference on Harmonization (ICH) standards. The technique exhibited linearity for both LAM and ZID within the 5–25 µg/ml concentration range, with correlation coefficients around 1. Precision studies demonstrate excellent repeatability with little intra- and inter-day variability, shown by low % RSD values. Results from recovery trials ranging from 98% to 102% demonstrated accuracy. The approach demonstrated sensitivity despite the moderate LOD and LOQ values. We exhibited our system's stability and consistency under many conditions throughout the robustness testing. The analysis of tablet formulations indicated that both medications were accurately quantified. The novel UV spectrophotometric technique provides a straightforward, sensitive, and cost-effective means of concurrently quantifying ZID and LAM in pharmaceutical formulations. It is useful for the routine evaluation of tablet dosage forms and quality assessment.

Keywords: Lamivudine, Zidovudine, Validation,Simultaneous and UV spectrophotoscopy.

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INTRODUCTION

Infection with human immunodeficiency virus type 1 (HIV-1) is the primary etiology of acquired immunodeficiency syndrome (AIDS), a major contributor to death for millions globally in recent years. Treatment failure transpires within one year of commencing antiretroviral therapy owing to the virus's fast development of resistance to several anti-HIV medications and the variability of side effects associated with dose levels. In recent years, traditional methods for managing acquired immunodeficiency syndrome (AIDS) have been supplanted by combination therapy. AIDS is often managed with a regimen that includes one protease inhibitor (PI) and two nucleoside reverse transcriptase inhibitors (NRTIs). HAART, or highly active antiretroviral therapy, is now the most effective treatment for HIV. The integration of several kinds of antiviral medications extends patient longevity.[1–3]

The chemical formula of lamivudine, an antiviral medication derived from cytosine, is 1[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl].[4-5] Zidovudine, abbreviated as ZID, is a benzimidazole derivative of tetrahydrofuran-2-yl. The antiretroviral efficacy of 5-methyl pyrimidine-2,4(1H,3H)-dione[6-7] is shown in Figure 1. Patients infected with HIV have received treatment with combinations of reverse transcriptase inhibitors, including LAM and ZID. This treatment seeks to prevent or postpone the onset of AIDS, a critical medical illness that may lead to mortality. Patients with liver inflammation due to hepatitis B virus replication get reduced doses of LAM. ZID is an antiviral drug used for the treatment of HIV. ZID may aid pregnant women in decreasing the probability of transferring the virus to their foetus. These approaches rely on the assessment of the physical qualities of the compounds, including both particular and non-specific attributes[8–10].

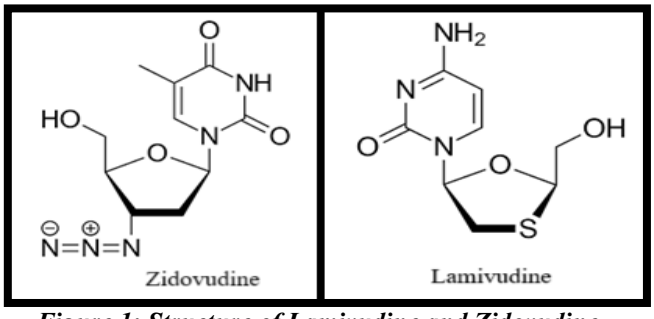

Figure 1: Structure of Lamivudine and Zidovudine

A literature analysis identifies many analytical approaches for the independent estimation of LAM and ZID using UV spectrophotometry. Moreover, there is a scarcity of RP-

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HPLC methodologies available for the individual or combined measurement of LAM and ZID with other pharmaceuticals[11–19]. A range of techniques has been used to estimate LAM in combination with ZID and other pharmacological agents[20–30].

Material and Method

The innovative method was evaluated using a UV-visible spectrophotometer (Shimadzu Corporation UV-1800 model) with appropriate 1-cm quartz cuvettes and automatic wavelength calibrations. The absorption spectra of the reference and test solutions throughout the 200–400 nm range were examined using a one-centimetre quartz cell. A Shimadzu AUX220 electronic balance (Japan) was used to guarantee accurate sample measurement. The tablet sample solution underwent sonication with an ultrasonic cleaning.

Reagents and chemicals

All chemicals and substances used were of analytical reagent grade. The reference standards for pure medicines, LAM and ZID, were supplied as complimentary samples by Danish labs in Ujjain. The whole research was performed with a phosphate buffer at pH 6.8.

Method development

Selection of solvent

Dissolving ZID and LAM requires a 6.8 pH phosphate buffer. Final decisions were based on the solvent's solubility of the standards and samples, the method's appropriateness, and the cost. Because it was inexpensive, non-toxic, and had the right solubility properties for the two active compounds, this investigation employed a phosphate buffer (pH 6.8)[31–32].

Selection of wavelength

A solution containing 10 µg/ml of ZID and another solution containing 10 µg/ml of LAM were both produced. We looked at the 200-400 nm range of both solutions' spectra. At 271.0 nm, the absorbance of LAM was recorded, whereas that of ZID was measured at 267.0 nm. Up to their respective maximums, ZID and LAM both showed linearity within the concentration range of 5-25 µg/ml. The relationship between absorbance and concentration was plotted to create a calibration curve[33–34].

Working solutions

Stock solution for LAM and ZID

In a 10-milliliter volumetric flask, 10-milligrams of each drug was dissolved in 8 millilitres of phosphate buffer (pH 6.8) to create the standard stock solutions. In order to get a concentration of 1000 µg/ml (Stock-A), the two medications were dissolved in the vial by subjecting it to sonication for about 10 minutes. The volume was then increased to 10 ml by adding 6.8 pH phosphate buffer. Furthermore, using a pipette, 1 ml portions of the LAM and ZID standard stock solutions A were transferred into individual 10 ml volumetric flasks. In order to get 100 µg/ml (Stock-B) of concentration, 10 ml of phosphate buffer at pH 6.8 was added to each vial.

Working Standard Solution

To make the sub stock solution (Stock-B), various volumes of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml were transferred into individual 10 ml volumetric flasks. Then, to get the volume down to 10 millilitres, phosphate buffer (pH 6.8) was added. The results showed that the concentrations of the ZID and LAM solutions were 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml, equally.

Preparation of Sample Solution

To get 10 milligrams of zidovudine, 20 tablets must be dissolved in 10 millilitres of solvent. Mill each pill to a fine powder subsequent to ascertaining its average weight. The commercially available pills, ZIDOLAM pills and Duovir capsules, had 5 milligrams of lamivudine. The flask underwent sonication for around 10 minutes after the dissolution of the medication in the tablet formulation with 5 millilitres of phosphate buffer (pH 6.8). The volume was then restored with buffer. The solution was filtered using Whatman filter paper No. 41 after sonication. Upon collection of the filtrate, the final concentrations of both drugs were diluted with buffer to guarantee they fell within the working range. The concentrations were measured by measuring the absorbance of the final dilutions at different wavelengths using the simultaneous equation method[35-37].

Procedure

Study of Overlay Spectra

The two medications' spectra were captured in spectral mode spanning the 200-400 nm range after scanning a standard stock solution containing 10 µg/ml of LAM and 10 µg/ml of ZID in a phosphate buffer with a pH of 6.8. At 267.0 nm, the absorbance peak of ZID was observed, while at 271.0 nm, the peak of LAM was detected. At 260.0 nm, the overlapped spectra likewise showed isoabsorptive regions. The simultaneous equation approach relies on the wavelength at which one medication (X or Y) is absorbed to the greatest extent, in relation to the wavelength at which the other drug is absorbed. The process displays the λmax values for each molecule using the two wavelengths: 271.0 nm for LAM and 267.0 nm for ZID. The absorptivities (A1%, 1 cm) of the two compounds were calculated by averaging five separate absorbance measurements taken at the given wavelengths. The following formulae were used to determine the sample concentrations:

$$Cx = \frac{A2\ ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

The mixture's absorbance at 271.0 nm is represented by A1, while the ZID's absorbance at 267.0 nm is represented by A2. At λ1 (271.0, the λmax of LAM) and λ2 (267.0, the λmax of ZID), the absorptivities of LAM are symbolically represented as Ax1 and ax2, respectively. The LAM concentration is represented by CX, while the ZID concentration is represented by CY.

Preparation of Calibration Curve

Linearity testing at 271.0 nm for LAM and 267.0 nm for ZID were used to define the operating concentration range. A standardized stock solution of ZID and LAM was prepared using water. The drug absorption velocities were determined by use of an equation.

$$\mathbf{A = abc}$$

Where, A=Absorbance, a = Absorptivity, b = Pathlength, c = Concentration.

Validation of spectroscopic methods

Implemented in accordance with the standards set out by the "International Conference on Harmonization," the previously developed protocols were verified as authentic.

Linearity

Using 10-milliliter volumetric flasks, the LAM and ZID standard solutions were divided into the corresponding volumes. In order to get the necessary concentrations of 5, 10, 15, 20, 25, and 30 µg/ml for LAM and ZID, the portions were diluted with phosphate buffer at a pH of 6.8. Calibration curves were generated by graphing absorbance versus concentration, which allowed for the establishment of regression equations for each drug. According to Beer-Lambert's law, the concentration ranges for ZID and LAM analysis was determined to be 5 to 30 µg/ml[38].

Accuracy

Analytical recovery experiments were conducted using the traditional addition method at 80%, 100%, and 120% concentrations to assess the efficacy of the established methods and the influence of pharmaceutical excipients. To calculate the percentage recovery, the total amount of medicines collected was used. There was a wide range of 98% to 102% recovery for ZID and LAM. The investigational results were obtained using the composition's standard proportions of LAM and ZID, and their reliability was evaluated using the following formula[39,40].

$$\% \text{ Recovery} = (\text{Amount of medication obtained after adding a standard drug quantity of medication discovered before adding a standard drug}) / (\text{quantity of a standard medication added}) \times 100.$$

Precision

The procedure's accuracy was assessed by investigations into intraday precision, inter-day precision, and repeatability. To evaluate the reproducibility of the techniques, we performed five separate tests on 10 µg/ml samples of ZID and LAM. The absorbance's of the drug solutions at 271.0 nm and 267.0 nm were quantified using SE techniques. To determine the RSD %, three independent studies were performed on the same day at two different concentration levels to assess intraday precision. The sample solutions (LAM and ZID) underwent testing at doses of 5, 10, 15, 20, and 25 g/ml. The RSD % was then calculated. The relative standard deviation (RSD) % was determined during three days of triplicate examination of sample solutions (LAM and ZID: 5, 10, 15, 20, and 25 g/ml) at two separate concentration levels within the linear range[41–42].

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection (LOD)

The minimal amount of analyte detectable in a sample is referred to as the detection limit of the analytical procedure. Consequently, it is not always feasible to ascertain a precise monetary worth.The expression for the LOD

$$LOD = \frac{3.3\ xSD}{Slope}$$

where σ = Response's relative standard deviation. S = The calibration curve's slope.

Limit of quantification (LOQ)

The lowest analyte concentration in a sample that can be determined with high accuracy and precision is known as the quantitation limit.One way to represent LOQ

$$LOQ = \frac{10\ xSD}{Slope}$$

where σ = Response's relative standard deviation, S = The calibration curve's slope (pertaining to the analyte).

Analysis of marketed formulation

For Marketed Formulation

Dissolve 20 tablets in 10 millilitres of water to get 10 milligrams of zidovudine. Once the average weight of each capsule has been determined, grind it into a fine powder. There were 5 milligrams of lamivudine in these pills that were sold in pharmacies (Duovir pills, ZIDOLAM Tablets). After adding 5 millilitres of phosphate buffer at a pH of 6.8, the medicine in the tablet formulation was dissolved in the flask using sonication for around 10 minutes. The volume was subsequently updated to incorporate the buffer. Following sonication, Whatman filter paper No. 41 was used for filtering. By collecting the filtrate and then diluting it with buffer, the final concentrations of the two medications were brought to the range that was suitable for use. The concentrations were determined using the simultaneous equation method, which entails taking absorbance readings at certain wavelengths for the final dilutions.

Result

A method for simultaneous estimate using UV spectra was devised and assessed for ZID and LAM. This procedure is straightforward, precise, and reliable. The concurrent estimation method was used to evaluate both pharmaceuticals.

Method optimization strategy

This work seeks to develop and assess a UV approach for the concurrent estimate of LAM and ZID via several experimental trials using different solvents

Selection of solvent and wavelength

We ran some preliminary experiments with different mixes of organic solvents to find out what worked best. In Trial 1, when distilled water was used as the diluent, LAM did not exhibit any appreciable absorbance. When 0.1 HCL was added to distilled water in Trial 2, ZID did not show any detectable absorbance or peak. Trial 3's 4.5 phosphate buffer with distilled water diluent showed no appreciable absorbance for LAM and ZID. A 6.8 pH phosphate buffer solution in distilled water was used as the diluent in the fourth attempt. A wavelength of 271 nm was used to test the LAM sample's absorbance. At 267 nm, the ZID showed a significant absorption. We found that the best solvent for simultaneous LAM detection was a buffer solution of 6.8 pH in distilled water. There was no interference between the 271 nm and 267 nm wavelengths. The diluent buffer pH was 6.8, and the LAM and ZID spectra were shown in Figure 2 in distilled water.

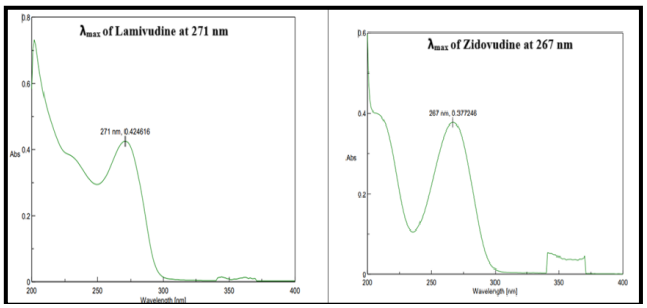

Figure 2: λmax of Lamivudine and Zidovudine

Study of overlay spectra

In order to gather data, the LAM and ZID spectra were finally superimposed. The simultaneous estimation approach was used to produce ZID data at 267 nm and LAM data at a peak wavelength of 271 nm.

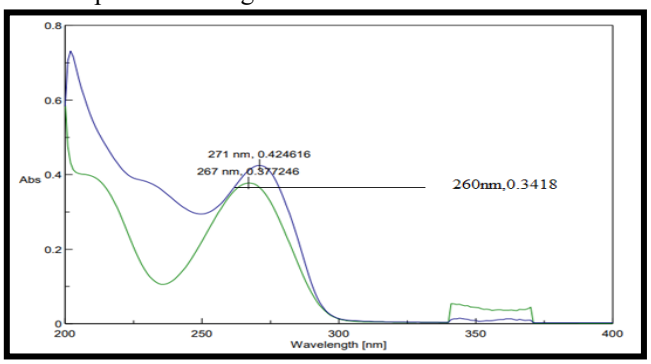

Figure 3: Overlain spectra of drugs (isoabsorptive points)

Preparation of calibration curve

The linearity test of LAM and ZID was carried out at a concentration range of 5-30 µg/ml at λmax 271.0 nm and 267 nm, respectively. There was less than a one-standard-deviation in the absorbance readings across all five concentration repeats. Strong linearity between concentration and absorbance was shown by the calibration curve's excellent correlation coefficient (r^2 = 0.998 & 0.995). The intercept is 0.000 and the slope is 0.026, with a standard deviation of 0.053.

Table 1: Results of Linearity of Drugs

Parameters	LAM	ZID
λmax (nm)	271	267
Concentration (µg/ml)	5 - 30	5 - 30
Correlation Coefficient (r^2) *	0.998	0.995
Slop (m) *	0.053	0.026
Intercept (c) *	0.000	0.029

*Value of five replicate

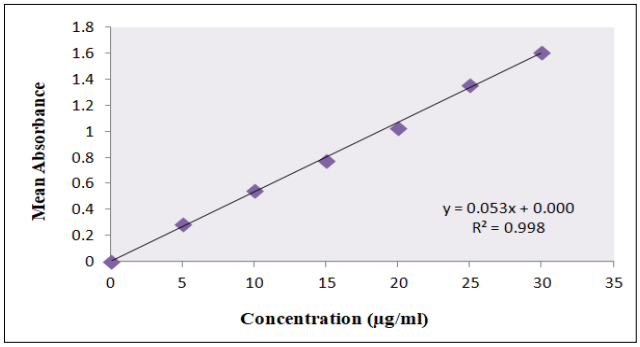

Figure 4: Calibration Curve of LAM

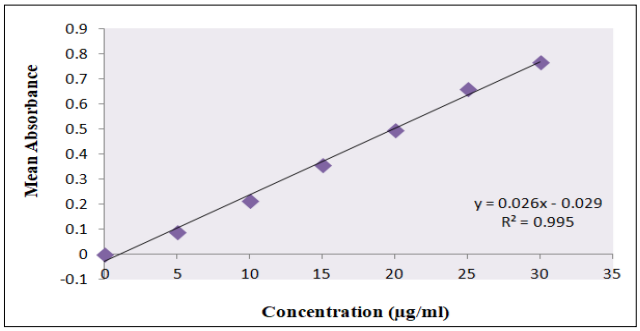

Figure 5: Calibration Curve of ZID

Validation of spectroscopic methods

Linearity

Analytical procedures are deemed linear if they provide findings that are proportionate to the analyte concentration in the sample within a defined range. A linear regression analysis was conducted to examine the relationship between concentration and absorbance. Within the 5 to 30 µg/mL range, the linear regression equations and R^2 values were 0.053x + 0.000 (R^2 = 0.998) for LAM and 0.026x - 0.029 (R^2 = 0.995) for ZID, demonstrating highly linear responses.

Table 2: Response Ratio of LAM and ZID

S. No.	LAM			ZID		
	Conc. (µg/ml)	ABS	Response Ratio	Conc. (µg/ml)	ABS	Response Ratio
1	5	0.2853	0.05706	5	0.0906	0.01812
2	10	0.5433	0.05433	10	0.2147	0.02147
3	15	0.7745	0.05163	15	0.3558	0.02372
4	20	1.0236	0.05118	20	0.4947	0.02474
5	25	1.3553	0.05421	25	0.6608	0.02643
6	30	1.6101	0.05367	30	0.7675	0.02558

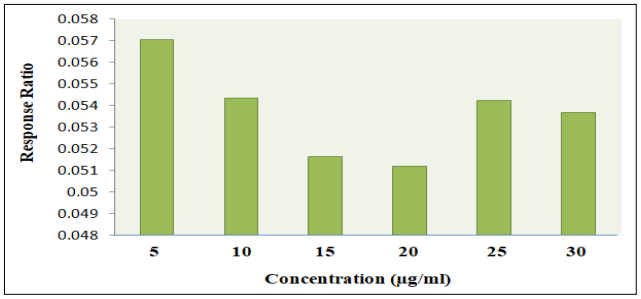

Figure 6: Graph of Response Ratio of LAM

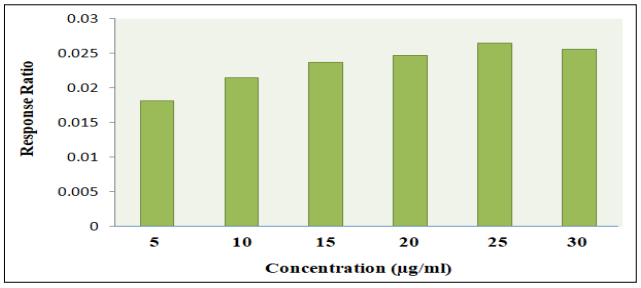

Figure 7: Graph of Response Ratio of ZID

Accuracy

The research performed recovery assessments at three specific levels: 80%, 100%, and 120% to validate the accuracy of the proposed methodologies. To achieve a standard equivalent square, the specified procedure required the incorporation of a certain amount of standard LAM and ZID at three tiers. Following an investigation of the contents, the mathematical recovery methods were calculated. The table below presents the mean recovery rates for LAM and ZID. The recovery levels fell within the acceptable range, and the outcomes were praiseworthy.

Table 3: Results of Recovery study of LAM & ZID

Sr. no.	Recovery level %	LAM Mean	S.D	% RSD	ZID Mean	S.D	% RSD
1	80	98.91	0.334	0.337	98.44	0.889	0.903
2	100	98.75	0.435	0.441	98.46	1.197	1.216
3	120	98.48	1.125	1.142	98.97	0.451	0.456

*Mean of 3 replicate and 5 concentrations

Precision

Intraday Precision

The repeatability investigations were conducted at dosages of 5, 10, 15, 20, and 25 µg/mL to confirm the procedures according to their accuracy. The results of repeatability tests provide light on the daily accuracy achievable, as well as studies or measurements carried out under continuous operating conditions for short periods. To find the methods' intraday accuracy, we measure the absorbance of a single sample five times in a single day. Variance, standard deviation, and relative standard deviation (% RSD) calculations are performed on the LAM and ZID variables. Findings regarding intraday accuracy for both LAM and ZID are shown in Table 4. With an RSD of less than 2%, both LAM and ZID demonstrate outstanding repeatability.

Interday precision:

The processes' accuracy was confirmed using repeatability tests conducted at 5, 10, 15, 20, and 25 µg/mL dosages. They provide light on the accuracy that can be achieved via short-term measurements or tests conducted under same conditions, as well as the precision that can be achieved across days. By taking readings of a consistent sample's absorbance on five different days (days 1, 2, 3, 4, and 5), we may assess the methods' interday accuracy. We then determine the LAM and ZID variables' standard deviations, coefficients of variation, and relative standard deviations (% RSD). The precisions for ZID and LAM between days are shown in Table 4. Since both ZID and LAM have relative standard deviations (% RSD) of 2 or less, it is clear that the suggested procedures are very accurate and reproducible.

Table 4: Results of Precision

Parameters	Mean (% conc)	SD	% RSD (% conc)	Mean (% conc)	SD	% RSD (% conc)
	LAM			ZID		
Repeatability	99.715	0.043	0.189	99.211	0.033	0.571
Day-to-Day variation	99.53	0.038	0.171	98.543	0.095	0.867
Analyst-to-Analyst Variation	99.45	0.150	0.475	98.035	0.107	1.003
Reproducibility	99.666	0.039	0.196	98.026	0.088	0.844

Limit of detection (LOD) and Limit of quantification (LOQ)

By determining the slope and standard deviation of their calibration curve, the Limit of Detection (LOD) and Limit of Quantification (LOQ) for ZID and LAM were determined. As an example, we can see the ZID limit of quantification (LOQ) and the LAM limit of detection (LOD) values. These processes are quite sensitive and exact, according to the findings.

Table 5: Results of LOD and LOQ

S. No.	LOD (µg/ml)	LOQ(µg/ml)
1	0.85	2.50
2	0.45	1.40

Analysis of marketed formulation.

As shown in Table 6, the concentration of each analyte was defined using the simultaneous equation approach.

Table 6: Assay by simultaneous equation method

Name of Formulation	Conc. Present (mg)		Conc. Found (mg)		% Conc. Found		%RSD	
	LAM	ZID	LAM	ZID	LAM	ZID	LAM	ZID
DUOVIR Tablets	150	300	149.74	298.85	99.827	99.617	0.255	0.341
ZIDOLAM Tablets	150	300	148.65	296.74	99.100	98.913	0.145	0.228

The results of the research have important implications for pharmacological analysis, especially when it comes to HIV therapy. An innovative UV spectrophotometric technique has been developed to detect LAM and ZID simultaneously; this approach is a valuable tool for research into pharmaceutical formulations. A gap in the current literature is filled by this approach. Speeding up the analytical process, allowing for the simultaneous examination of both active components improves quality control and regulatory compliance. This approach is great for regular testing in regulatory labs and pharmaceutical businesses since it is easy to use, cheap, and very sensitive. Evidence from the present suggests that the proposed approach enhances analytical methods used in the pharmaceutical sciences. While earlier studies provided methods for evaluating ZID and LAM independently, the current work presents the first way for evaluating combination pill formulations including both drugs at the same time. A more thorough examination of pharmacological mixtures is made possible, and the tools for evaluating combination medicine quality are enhanced. The research did have a number of limitations, however, and that must be taken into consideration. To evaluate the technique's effectiveness across a wider variety of experimental settings and pharmaceutical formulations, more validation studies could be necessary. Additional study might shed light on how well this strategy works in actual pharmaceutical manufacturing environments and how it applies to other medication combinations. Innovative UV spectrophotometric technique outperforms conventional approaches to pharmaceutical analysis. Its dependability, effectiveness, and adaptability make it a potentially useful tool for the pharmaceutical business to use in guaranteeing product quality and regulatory compliance.

Discussion

The results of the research have important implications for the assessment of pharmaceuticals, particularly those used to treat HIV. An important gap in the literature has been filled and a powerful tool for investigating pharmaceutical formulations has been developed: a UV spectrophotometric method for the simultaneous assessment of LAM and ZID. By enabling the simultaneous analysis of both active components, this technique streamlines the analytical procedure. Compliance with regulations and quality control are both made easier by this. The low cost, ease of use, and high sensitivity of this technology make it an essential tool for regulatory labs and pharmaceutical corporations to use for regular testing. By factoring in current research, the suggested technique enhances analytical procedures in the pharmaceutical sciences. Even while other studies have developed independent calculation procedures for ZID and LAM, this work presents a better way for their simultaneous analysis in combination pill formulations. This allows for a more thorough examination of pharmacological compositions and increases the resources available for evaluating the quality of combination medications.Having said that, the study's shortcomings must be recognized. To evaluate the method's performance with a broader variety of pharmacological formulations and experimental settings, more validation trials could be necessary. The method's potential for use in the pharmaceutical industry and its relevance to complementary and alternative medicine integrations are potential areas for further study. All things considered, the new UV spectrophotometric technique is a giant leap forward for pharmaceutical examination. Due to its dependability, efficiency, and adaptability, it is a valuable instrument for evaluating the operational effectiveness of the pharmaceutical industry and guaranteeing compliance.

Conclusion

The results of the recovery study showed that the method successfully detected minute variations in the drug concentration in the solution. The technique demonstrated increased sensitivity, as seen by the lowered LOD and LOQ. The proposed method was confirmed to meet the standards set by the ICH. The suggested methods exhibit linearity, precision, specificity, and reproducibility in addition to being efficient and easy to execute. Whether in tablet or pure form, they may be used to concurrently identify ZID and LAM. When it comes to routinely assessing and evaluating tablet dosage forms, the proposed approach provides a unique, accurate, sensitive, and cost-effective option..

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