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Research Article

A New Analytical *Q*-Absorbance Ratio Method Development and Validation for Simultaneous Estimation of Lamivudine and Isoniazid

Gitu Pandey and Brahmeshwar Mishra

Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi Uttar Pradesh 221005, India

Correspondence should be addressed to Brahmeshwar Mishra; bmishrabhu@rediffmail.com

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A new UV spectrophotometric absorption ratio method was developed and validated for the simultaneous estimation of lamivudine and isoniazid. The method involved *Q*-absorption ratio analysis using two wavelengths, with one being the λ_{max} of lamivudine (272 nm, λ_2) and the other being the isoabsorptive point of both drugs (246 nm, λ_1). Beer's law was obeyed in the concentration range between 5 and 30 μg/mL for both lamivudine and isoniazid. The results of analysis have been validated statistically and by recovery studies as per ICH guidelines. The accuracy ranged between 99.65 and 101.91% and Sandell's sensitivity ranged between 0.0229 and 0.0347 μg/cm^2. The method was found to be simple, precise, reproducible, rapid, and economical. Hence, it could be used in the analysis of laboratory samples and marketed formulations containing these two drugs in the future.

1. Introduction

A recent WHO report on tuberculosis (TB) in 2012 has shown that there were an estimated 8.7 million incident cases of TB in 2011 (13% coinfected with HIV). There were also 1.4 million deaths from TB, 990,000 deaths among HIV-negative individuals and 430,000 among people who were HIV-positive [1]. Many TB carriers who are infected with HIV are 30 to 50 times more likely to develop active TB than those without HIV [2]. HIV infected individuals are not only at a greater risk for acquiring TB but also reactivation of latent TB infection is greatly increased due to the fact that the very cells that hold the latent TB in check (the CD4+ T lymphocytes) are precisely the cells that are rendered dysfunctional in HIV-infected individuals. There are evidences to believe that the main factor for the resurgence of TB has been the human immunodeficiency virus (HIV) [3]. Lamivudine (LAM), a leading antiretroviral drug, also known as 3TC, is chemically 2(1*H*)-pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-, (2R-cis) (Figure 1) with molecular formula $C_8H_{11}N_3O_3S$ and molecular weight 229.26 [4]. This deoxycytidine analogue is phosphorylated intracellularly and inhibits HIV reverse transcriptase as well as hepatitis B virus DNA polymerase. Most human DNA polymerases are not affected and systemic toxicity of 3TC is low [5]. Isoniazid (INH), a first line antitubercular, is chemically 4-pyridinecarboxylic acid hydrazide or isonicotinic acid hydrazide (Figure 2), having molecular formula $C_6H_7N_3O$ and molecular weight 137.14 [4]. It acts by inhibiting the synthesis of mycolic acids which get attached to arabinogalactan to form part of mycobacterial cell wall. It is an essential component of all antitubercular regimens, unless the patient is not able to tolerate it or bacilli are resistant [5, 6]. The simultaneous analysis of these two drugs INH and LAM is highly desirable as this will allow more efficient clinical data generation in the patients who are coinfected with tuberculosis and AIDS and for quantitative estimation of these drugs in combination formulations which may be marketed in the future. The absorbance ratio method is a method for simultaneous estimation of two components depending upon the property that the ratio of absorbances at any two wavelengths is a constant value independent of concentration or pathlength [7, 8]. An extensive and intensive literature survey has revealed that there is no

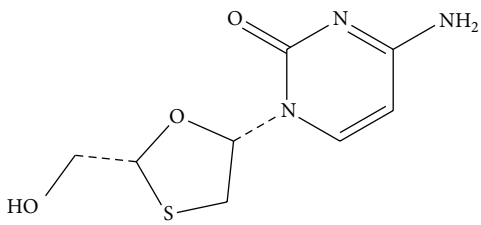

Figure 1: Structure of lamivudine.

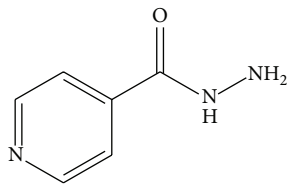

Figure 2: Structure of isoniazid.

absorption ratio method for simultaneous analysis of LAM and INH in pharmaceutical preparations. However it has been used for simultaneous analysis of prednisolone and 5-amino salicylic acid, valsartan, and hydrochlorothiazide, metformin hydrochloride, and fenofibrate [9–11]. The present work describes a simple, accurate, and precise absorption ratio method for simultaneous determination of these two drugs. The method was validated as per the current ICH guidelines [12–14].

2. Materials and Methods

2.1. Reagents and Apparatus. Lamivudine and isoniazid were obtained as gift samples from Mylan Laboratories, Nashik, Maharashtra, India, and Lupin Pharma Ltd., Pune, Maharashtra, India, respectively. All other chemicals used were of analytical grade. A double beam UV-Visible spectrophotometer, model 1700, Shimadzu, Japan, with software UV Probe 2.10 and 1 cm quartz cell, was used for all analysis.

2.2. Preparation of Standard Stock Solutions. Standard stock solution (1000 μg/mL) of LAM and INH was prepared separately by dissolving carefully weighed 100 mg of drug in 100 mL volumetric flask and diluting up to the mark with phosphate buffer (pH 7.4). Ten mL of this solution was diluted up to 100 mL with phosphate buffer (pH 7.4) to get working stock solution (100 μg/mL).

2.3. Determination of Isoabsorptive Point and Wavelength of Maximum Absorbance (λ_{max}). Solutions of 10 μg/mL of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against phosphate buffer (pH 7.4) as blank. The overlaying spectrum was also obtained to determine isoabsorptive point.

2.4. Preparation of Sample Solutions from Standard Stock Solution. The sample solutions of various concentrations were prepared from the standard stock solution by diluting aliquots of working stock solutions appropriately.

2.5. Calibration Curve (Linearity). A calibration curve was plotted over a concentration range of 5–30 μg/mL for both LAM and INH. Accurately measured working stock solution of LAM (2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mL) and working stock solution of INH (2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mL) were transferred to two separate series of 50 mL volumetric flask and diluted up to the mark with phosphate buffer (pH 7.4). The absorbance of both solutions was taken at their respective λ_{max} and at isoabsorptive point. The calibration curves were constructed by plotting concentration against absorbance where each reading was an average of three determinations.

2.6. Application of the Proposed Method for Estimation in Standard Laboratory Mixture. The absorptivity coefficient of both drugs was determined and the individual concentration of LAM and INH was determined using the following equations:

$$C_{\mathrm{INH}} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{X1}},$$
$$C_{\mathrm{LAM}} = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_1}{a_{Y1}}, \quad (1)$$

where $Q_M = A_2/A_1$, $Q_X = a_{X2}/a_{X1}$, and $Q_Y = a_{Y2}/a_{Y1}$; A_1 and A_2 are the absorbance, of the mixture at 246 nm and 272 nm, respectively; a_{X1} and a_{Y1} are absorptivities of INH and LAM, respectively, at 246 nm; a_{X2} and a_{Y2} are absorptivities of INH and LAM, respectively, at 272 nm.

3. Method Validation

3.1. Linearity and Range. Linearity, consisting of the basic elements input → converter → output, is the assumption that there is a straight line relationship between the input (x) and output (y) variables that can be written mathematically by the expression $y = f(x)$ if the straight line crosses through the origin or by the expression $y = f(x) + \delta$ if the straight line does not cross through the origin. The linear range corresponds to the valid interval of functional dependence of the signal on concentration or mass which assumes homoscedasticity of the measurements over the linear range. The linear response of LAM and INH was determined by analyzing five independent levels of the calibration curve in the range of 5–30 μg/mL.

3.2. Precision. The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions [11]. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation.

3.3. Repeatability (Within-Run Precision). It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of LAM and INH (15 + 15 μg/mL) was prepared and analyzed six times as per the proposed method.

3.4. Intermediate Precision (Between-Run Precision). It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution of LAM and INH (15 + 15 μg/mL) was prepared and analyzed as per the proposed method.

3.5. Accuracy (% Recovery). The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery experiments were carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards.

3.6. Limit of Detection (LOD) and Limit of Quantification (LOQ). The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$\text{LOD} = \frac{3.3\sigma}{S}, \qquad \text{LOQ} = \frac{10\sigma}{S}, \tag{2}$$

where σ is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve.

3.7. Sandell's Sensitivity. Sandell's sensitivity, the concentration of the analyte (in μg/mL or μg/cm^2) which will give an absorbance of 0.001 in a cell of path length 1 cm, was calculated. It gives valuable information regarding sensitivity of the method.

4. Results and Discussion

The solutions of 10 μg/mL of both LAM and INH were analyzed and the λ_{max} was found to be 272 nm and 262 nm, respectively. Three isoabsorptive points: 246 nm, 257 nm, and 292 nm were found in overlaying spectra (Figure 3) and the isoabsorptive point 246 nm was selected for further analysis.

The calibration curve of LAM and INH individually and the mixture of both drugs at 272 nm (λ_1) and 246 nm (λ_2) were plotted (Figures 4, 5, and 6). The relationship between the absorbance and the concentration of LAM and INH was found to be linear in the range of 5–30 μg/mL at

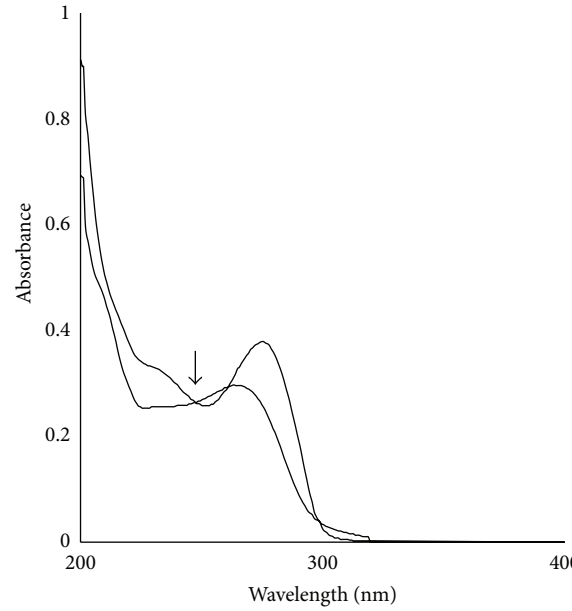

Figure 3: UV scan of LAM and INH showing isoabsorptive points.

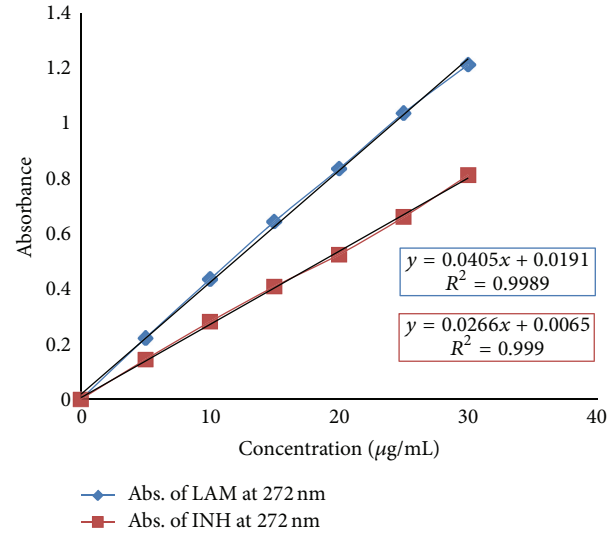

Figure 4: Calibration curve of LAM and INH at 272 nm.

both wavelengths 246 nm and 272 nm. The representative linear equations were calculated by the least squares method and the correlation coefficients have indicated very good linearity (Table 1). Evaluation of repeatability and intermediate precision was done and coefficients of variation (CV) or percent relative standard deviation (%RSD) values were calculated. These values were found to be less than two (CV < 2), indicating good precision (Table 2). Good accuracy of the proposed method was proved by good percent recovery in standard addition method. It ranged between 99.65 and 101.91% for LAM and 101.26 and 100.12% for INH (Table 3).

The limit of detection of LAM and INH at isoabsorptive point (246 nm) was found to be 0.106 μg/mL and 0.078 μg/mL. The LOD at 272 nm was found to be

Table 1: Calibration points of standard curve with standard deviation (SD) and %RSD.

Concentration of the solution (μg/mL)	At 246 nm				At 272 nm			
	LAM		INH		LAM		INH	
	Mean absorbance ± SD (n = 3)	%RSD	Mean absorbance ± SD (n = 3)	%RSD	Mean absorbance ± SD (n = 3)	%RSD	Mean absorbance ± SD (n = 3)	%RSD
5	0.152 ± 0.0015	1.007159	0.149 ± 0.0020	1.400224	0.222 ± 0.0025	1.132	0.144 ± 0.0026	1.83733
10	0.288 ± 0.0026	0.918664	0.287 ± 0.0025	0.875851	0.435 ± 0.0026	0.608	0.281 ± 0.0049	1.75756
15	0.429 ± 0.0040	0.941332	0.419 ± 0.0040	0.965315	0.645 ± 0.0035	0.544	0.409 ± 0.0040	0.98894
20	0.569 ± 0.0035	0.617565	0.538 ± 0.0036	0.670177	0.836 ± 0.007	0.837	0.525 ± 0.0045	0.85945
25	0.715 ± 0.0046	0.64092	0.687 ± 0.0057	0.828093	1.038 ± 0.0060	0.581	0.661 ± 0.0065	0.98383
30	0.834 ± 0.0055	0.660116	0.802 ± 0.0060	0.751273	1.214 ± 0.0095	0.786	0.813 ± 0.0035	0.43214

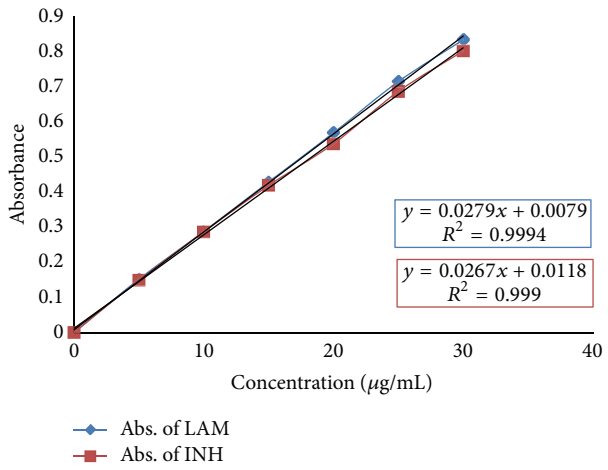

Figure 5: Calibration curve of LAM and INH at 246 nm.

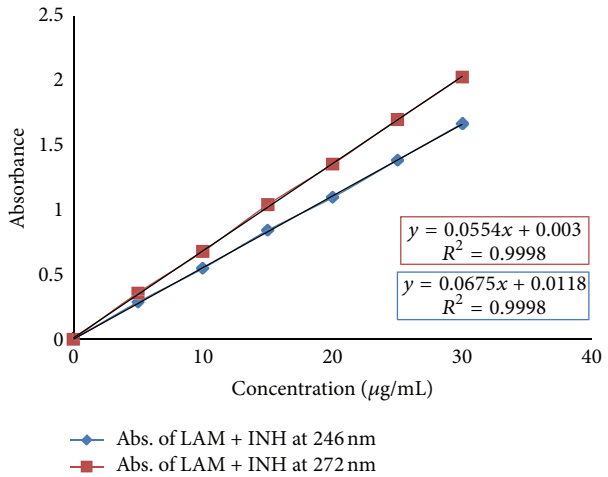

Figure 6: Calibration curve of LAM + INH at 272 nm and 246 nm.

0.186 μg/mL and 0.211 μg/mL for LAM and INH, respectively. The limit of quantification of LAM and INH at isoabsorptive point (246 nm) was found to be 0.321 μg/mL and 0.238 μg/mL. The LOQ at 272 nm was found to be 0.563 μg/mL and 0.639 μg/mL for LAM and INH, respectively. Sandell's sensitivity of LAM and INH at 246 nm was found to be 0.0347 and 0.0348 μg/cm^2. At 272 nm it was 0.0229 and 0.0356 μg/cm^2 for LAM and INH, respectively, which indicates good sensitivity of the method. Various validation parameters have been summarized in Table 4.

Table 2: Intraday and intermediate precision study.

Precision	% Estimation of LAM ± SD (n = 6)	%RSD	% Estimation of INH ± SD (n = 6)	%RSD
Intraday precision	99.88 ± 0.56	0.562	99.92 ± 0.84	0.844
Intermediate precision	99.79 ± 0.53	0.531	99.82 ± 0.86	0.861

Table 3: Results of recovery studies of LAM and INH.

Drug	Amount taken (μg/mL)	Amount added (μg/mL)	Amount found (μg/mL)	% Recovery ± SD (n = 3)
INH	10	1	11.14	101.26 ± 0.40
	10	2	12.02	100.17 ± 0.87
	10	3	13.02	100.12 ± 0.98
LAM	15	1	16.31	101.91 ± 0.68
	15	2	17.15	100.90 ± 0.57
	15	3	17.93	99.65 ± 1.23

5. Conclusion

The UV spectrophotometric Q-absorption ratio method was developed and validated for the simultaneous analysis of LAM and INH. The results together established that the method is simple, accurate, precise, reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of LAM and INH in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

Table 4: Summary of regression characteristics and validation parameters.

Parameters	LAM		INH	
	246 nm	272 nm	246 nm	272 nm
Beer's law limit (μg/mL)	5–30	5–30	5–30	5–30
Absorptivity	287	435	288	276
Regression equation ($y = mx + c$)				
Slope (m)	0.0279	0.0405	0.0267	0.0266
Intercept (c)	0.0079	0.0191	0.0118	0.0065
Correlation coefficient (r^2)	0.9994	0.9989	0.999	0.999
Standard deviation (SD)	0.0009	0.0023	0.0006	0.0017
LOD (μg/mL)	0.106	0.186	0.078	0.211
LOQ (μg/mL)	0.321	0.563	0.238	0.639
Sandell's sensitivity (μg/cm^2)	0.0347	0.0229	0.0348	0.0356

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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