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# SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF LAMIVUDINE AND ABACAVIR IN THE MIXTURE

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## ABSTRACT

A novel, simple, rapid and sensitive spectrophotometer method has been developed for simultaneous estimation of Lamivudine and Abacavir. The method employs formation and solving of simultaneous equation using 280 nm and 297 nm as two analytical wavelengths. Both the drugs obey Beer's Law in the concentration ranges employed for this method. Accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The method is found to be rapid, precise and accurate and can easily be employed in the laboratory for the routine estimation of drugs.

**INTRODUCTION:** Lamivudine (3TC) is a cytosine analog with potent activity against human immunodeficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity. Lamivudine is used in treatment of HBV infections and it has strongly been recommended for the treatment of HIV infections in combination with other antiviral drugs [1]. Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Abacavir is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis [2, 3].

Literature survey reveals several methods that have been used for the quantitative determination of the two drugs individually, such as for Lamivudine (LAMI) UV spectrophotometry, HPLC, estimation in pharmaceutical dosage forms by UV spectrophotometry and RP- HPLC and in plasma determination of LAMI by HPLC3-5 [4-6]. For Abacavir direct spectrometric assay, colorimetric estimation, in body fluids by TLC with fluorometric detection and HPLC with UV detection [7-11].

**MATERIALS AND METHODS:** Reference standard of lamivudine and abacavir were procured from Shreeyam Labs, Ahmadabad and Cipla Pharma, Rajkot respectively. Methanol (Qualigens, Mumbai), disodium hydrogen orthophosphate, potassium dihydrogen phosphate, sodium chloride were of AR grade and distilled water were used in the present study. JASCO double beam UV/Visible spectrophotometer (Model UV-530) with fixed slit width of 2 nm was used for experiment. Absorption and overlain spectra were recorded over the wavelength range of 200-400 nm, using 1cm quartz cells at a scan speed of 1200.00 and fixed slit width of 2.0 nm.

**Preparation of Standard Stock Solution:** Stock solutions (100µg/ml) of abacvir and LAMI were prepared by dissolving separately 10 mg of drug in 1 M HCl and making up the volume with 1 M HCl. The stock solution was suitably diluted to produce solution of concentration 10 µg/ml. These working solutions were scanned in the entire UV range (200-400 nm) to determine the $\lambda_{max}$.

Absorption maxima of lamivudine and abacavir were detected at 280 nm ($\lambda_1$) and 297 nm ($\lambda_2$), respectively and overlain spectra was recorded. A series of standard dilutions of each drug were prepared having concentration range of 2-12 µg/ml. Both LAMI and Abacavir showed linearity with absorbance in the range 2-12 µg/ml at their respective maxima. The absorbance was measured at 280 nm and 297 nm and calibration curves were plotted at these wavelengths.

**Recovery Studies:** Recovery studies were done so as to check the accuracy of the method. The accuracy of the method was assessed by taking known amounts of LAMI and abacavir in standard mixture solution and absorbance were determined at 280 nm and 297 nm. Concentration of the drugs in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates.

**RESULTS:**

**Absorption Maxima:** Absorption maxima of Lamivudine and Abacavir were detected at 280 nm ($\lambda_1$) and 297 nm ($\lambda_2$), respectively and overlain spectra was recorded (Fig. 1).

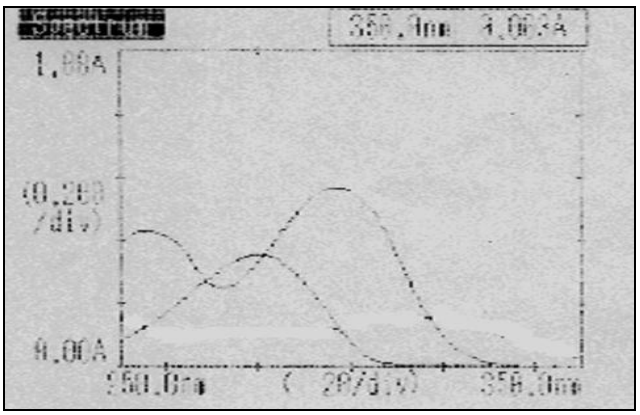

**FIG. 1: OVERLAIN SPECTRA OF LAMIVUDINE AND ABACAVIR**

**Absorptivity Coefficients:** The absorptivity coefficients of the two drugs were determined by using Beer's law: A = E (1%, 1cm) CL. The absorbptivity coefficients of lamivudine at 280 nm and 297 nm were 0.036283±0.003159 and 0.035567± 0.002815 and for Abacavir were 0.076733±0.00794 and 0.052233±0.026719. The observations are presented in Table 1. The optical characteristics and regression values for the calibration curve are presented in Table 2.

**TABLE 1: ABSORBPTIVITY VALUES FOR LAMIVUDINE AND ABACAVIR**

| CONCENTRATIONS (µg/ml) | | ABSORPTIVITY | | | |
|---|---|---|---|---|---|
| | | 280 nm | | 297 nm | |
| Lamivudine | Abacavir | Lamivudine | Abacavir | Lamivudine | Abacavir |
| 2 | 2 | 0.031 | 0.0317 | 0.092 | 0.0879 |
| 4 | 4 | 0.0345 | 0.0334 | 0.077 | 0.0752 |
| 6 | 6 | 0.0363 | 0.0349 | 0.069 | 0.0595 |
| 8 | 8 | 0.0375 | 0.0362 | 0.074 | 0.0416 |
| 10 | 10 | 0.0388 | 0.038 | 0.0752 | 0.0308 |
| 12 | 12 | 0.0396 | 0.0392 | 0.0732 | 0.0184 |
| MEAN | | 0.036283 | 0.035567 | 0.076733 | 0.052233 |
| SD | | 0.003159 | 0.002815 | 0.00794 | 0.026719 |

n=3

**TABLE 2: OPTICAL CHARACTERISTICS**

| CHARACTERISTICS | LAMIVUDINE | ABACAVIR |
|---|---|---|
| $\lambda_{max}$ | 280 | 297 |
| Beer's Law Limit (µg/ml) | 2-12 | 2-12 |
| Molar Absorptivity | 1608.667 | 4409.667 |
| Regression Equation | Y=0.3499x + 0.0399 | Y=0.3541 + 0.0575 |
| Slope | 0.3499 | 0.3541 |
| Intercept | 0.0399 | 0.575 |
| $r^2$ | 0.9956 | 0.999 |

n=3

**Partial simultaneous equation method:** A set of two simultaneous equations were framed using the mean absorptivity.

Coefficients values, as given below:-

At λ1 (280 nm) A1 = ax1bCx + ay1bCy
A1 = 0.036283Cx + 0.035567Cy
At λ2 (297 nm) A2 = ax2bCx + ay2bCy
A2 = 0.076733Cx + 0.052233Cy

Where A1 and A2 are absorbance at 280 nm and 297 nm, Cx and Cy are the concentration of LAMI and Abacavir respectively (µg/ml).

**Recovery studies:** Recovery studies were done so as to check the accuracy of the method. The analysis was done in a set of 3 replicates and results are summarized in Table 3.Recovery was close to 100% stating the accuracy and reproducibility of the method.

**TABLE 3: RECOVERY STUDIES**

| DRUG IN STANDARD MIXTURE (µg/ml) | | RECOVERY ± SD | |
|---|---|---|---|
| Lamivudine | Abacavir | Lamivudine | Abacavir |
| 4 | 4 | 99.77 | 99.94 |
| 6 | 6 | 98.68 | 100.70 |
| 8 | 8 | 100.66 | 100.41 |
| Mean | | 99.70 | 100.35 |
| ±S.D | | 0.5725 | 0.2214 |
| S.E. | | 0.3253 | 0.1258 |
| C.V | | 0.0057 | 0.0022 |

**DISCUSSION:** The validation parameters were studied at both the wavelengths for the method. Accuracy and reproducibility was determined by calculating the recovery that was close to 100%. Precision was calculated as repeatability (SD and %CV). The proposed method is simple, precise, accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis.

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