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DEVELOPMENT AND VALIDATION OF ANALYTICAL TECHNIQUE FOR ANTIRETROVIRALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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ABSTRACT

Drug analysis plays an important role in the development of drugs, their manufacture and therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and final products obtained meet the required specifications. The number of drugs and drug formulations introduced into the market has been increasing at an alarming rate. These drugs or formulations may be either new entities or partial structural modifications of the existing ones or novel dosage forms (controlled/ sustained release formulations) or multi component dosage forms. Very often, there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not available in pharmacopoeias. It becomes necessary, therefore, to develop newer analytical methods for such drugs. Considering all these views some drug formulations from Antiretrovirals were selected for the present study. An extensive literature survey was carried out and it is evident that methods like High Performance Liquid Chromatography (HPLC) have been reported for the estimation of these drugs in their biological fluids[15-18]. There are however, no reports for their estimation by HPLC in their formulations. It becomes essential, therefore, to develop newer rapid analytical methods by HPLC[1-14].

KEYWORDS: *Anti-retrovirals, analytical technique, development, validation, HPLC*

INTRODUCTION

DRUG PROFILE

LAMIVUDINE

Lamivudine is (2R-cis) –4amino-1(2-hydroxy methyl)-1,3-oxathiolon-5-yl) 2-(1H)-pyrimidinone(-)-2'-deoxy-3'-thiacytidine.

Molecular formula: $C_8H_{11}N_3O_3S$

Molecular weight: 229.26

White crystalline powder having melting point at 122^0C.

ZIDOVUDINE

Zidovudine is 1-(3-azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1H,3H)-dione.

Molecular formula: $C_{10}H_{13}N_5O_4$

Molecular weight: 267.2

A white or brownish powder, sparingly soluble in ethanol. It melts about 124^0C.

MATERIALS AND METHOD (EXPERIMENTAL)

ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE BY REVERSE PHASE HPLC METHOD

Instrument: HPLC (Waters)

Chemicals & Reagents:

Standard Zidovudine from Strides Arcolab limited Bombay & Lamivudine from CADILA at Ahmadabad were procured. The combination formulations were obtained as marketed drugs from the drug stores.

Mobile phase

Methanol HPLC grade, water HPLC grade were used. 50mM Potassiumdihydrogen phosphate (6.8gm) was dissolved in water (1000.0ml). Buffer (650.0ml) and of methanol (350.0ml) were mixed and filtered through 0.45µ filter paper and sonicated.

Standard preparation

10mg of standard drug was taken in 10ml standard volumetric flask and dissolved in the mobile phase using sonicator. And the stock solution was further diluted to micro gram level concentration with the mobile phase.

Linearity of detector response

Separate calibration curves were obtained, solutions were prepared by taking varying concentrations of Zidovudine (10µg to 50µg) and Lamivudine (10 µg to 30 µg) of Lamivudine separately by plotting graph Area vs. Concentration by this linearity of detector response was checked. **Figure 1 & 2**.

Figure 1-Linearity Response of Detector for Lamivudine

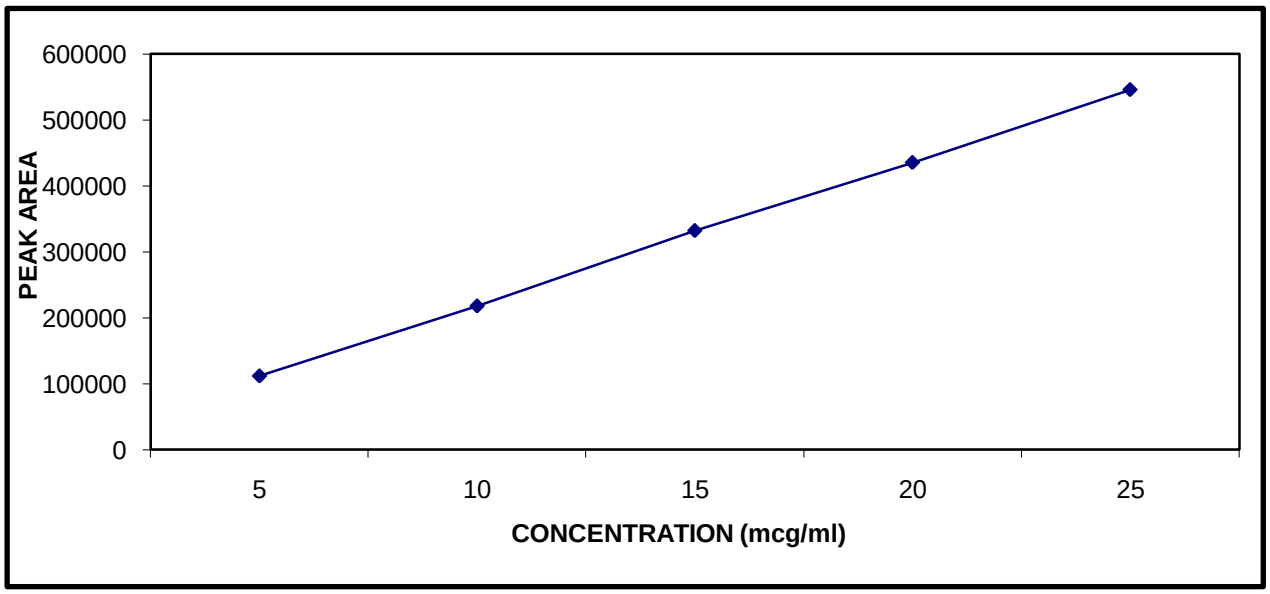
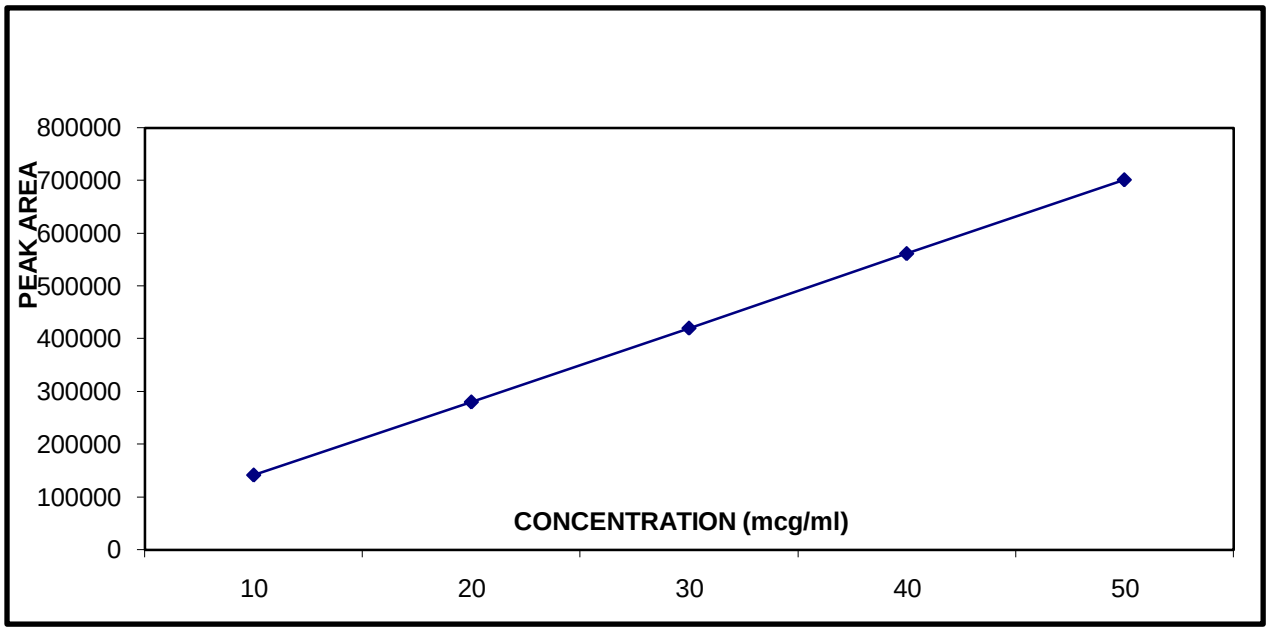


Figure 2-Linearity Response of Detector for Zidovudine



Chromatography: The flow rate was maintained at 1.2 ml/min. Temperature of the column (Thermo hypurity C18, 50*4.6mm,5µ) was ambient, the average pressure was 2100 psi and the effluents

were monitored at 221nm. The mobile phase used was Buffer and methanol (65:35).

Calibration curves were constructed for Lamivudine and Zidovudine by plotting the peak area of drug i.e. (y axis) against the concentration of drug µg/ml (x axis), **Figure 1&2**.

Experiment

Twenty tablets, each of combined dosage were accurately weighed and powdered. A fine composite quantity equivalent to 300 µg of Zidovudine and 150 µg of Lamivudine taken dissolved in 100ml of mobile phase and made the dilutions to obtain final concentration 30 µg of Zidovudine and 15 µg of Lamivudine.

Formula:

$$\%\text{Of Sample} = \frac{\text{Mean Area of Sample}}{\text{Mean Area of Standard}} \times \frac{\text{Weight of Standard}}{\text{Dilution Factor}} \times \frac{\text{Dilution Factor of Sample}}{\text{Weight of Sample}} \times \text{Label Claimed}$$

Recovery experiment

To study the accuracy, reproducibility and precision of the proposed method recovery experiments were carried out. A fixed amount of the pre analyzed sample was taken and standard drug was added at three different levels each level was repeated for 5 times. The summary of recovery is as given in **Table 3&4**.

RESULTS AND DISCUSSION

The present method is a high performance liquid chromatography method to determine Zidovudine and Lamivudine from its formulations, various experiments were carried out to separate them and mobile phase phosphate buffer and methanol in proportion of (65:35), is found to be ideal for the separation of this combination the elution was in the following order Lamivudine (RT/2.64 min) Zidovudine (RT/4.96). The mean recoveries of Zidovudine and Lamivudine were 100.01% and 100.05% respectively. The values of percent recovery and standard deviation shown that the proposed method is accurate, reproducible and precise. The summary of final results was given in **Table no.1&2**.

Table 1-Reproducibility experiment for Lamivudine

Sl. No	Name of company	Amount found Mg/ tablet + SD	%RSD	Percentage of Assay
1	LADIWIN (ZYDUS CADILA)	150.394 ± 0.86	0.34	100.26
2	LAMIVIR (cipla)	150.650± 0.85	0.61	100.43

Table 2- Reproducibility experiment for Zidovudine

Sl. No	Name of company	Amount found Mg/ tablet + SD	%RSD	Percentage of Assay
1	ZIDOWIN (ZYDUS CADILA)	300.232±0.37	0.13	100.07
2	ZIDOVIR (cipla)	300.436±0.46	0.23	100.14

Table 3-Recovery experiment for LAMIVUDINE

Label claim amount of std added in mg	Amount of standard drug added in mg	Amount recovered in mg	% of recovery
150mg	0.0	150.359	100.24
150mg	50	199.641	99.82
150mg	100	249.704	99.88
150mg	150	300.286	100.10

Table 4- Recovery experiment for ZIDOVUDINE

Label claim amount of std added in mg	Amount of standard drug added in mg	Amount recovered in mg	% of recovery
300	0	299.793	99.93
300	100	400.2	100.05
300	200	499.75	99.95
300	300	601.2	100.20

CONCLUSIONS

The accuracy of the method was noted and it was felt that method can be suitably adapted for other drugs and combination in further studies.

HPLC method has been accurate and it gives details with regards best separation and calculation of concentration simultaneously.

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