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Simultaneous estimation of lamivudine and zidovudine by RPHPLC

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ABSTRACT

A simple, accurate, rapid, reproducible HPLC method has been developed for simultaneous estimation of lamivudine and zidovudine using a mobile phase consisting 7.7gms of ammonium acetate and acetonitrile in the ratio of 93:7, 75:25 at a flow rate of 1.5ml/min. A hypersil BDS 150 x 4.6mm, C₁₈ column was used as a stationary phase. Quantification was performed using PDA detector at 270nm. The method showed good resolution between two peaks.

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KEYWORDS

RP-HPLC;
Lamivudine;
Zidovudine.

INTRODUCTION

Lamivudine enters cells by passive diffusion and is phosphorylated to its active metabolite, lamivudine triphosphate^[1]. It competes with deoxycytidine triphosphate for binding to reverse transcriptase and incorporation into DNA results in chain termination^[2-6]. Chemically it is designated as L-2', 3'- dideoxy-3'-thiacytidine.

Zidovudine converted to triphosphate derivative by the host cell thymidylate kinase. ZVD triphosphate is the active form, which competes with thymidine 5- triph-

osphate for binding to the HIV reverse transcriptase^[6-14]. Chemically it is designated as 3'-azido-2', 3'-di deoxythymidine.

The composition of lamivudine and zidovudine is used for treating HIV. An attempt has been made in this work to devise a simple and accurate HPLC method for simultaneous estimation of lamivudine and zidovudine.

ASSAY METHOD AND MATERIALS

Instrument

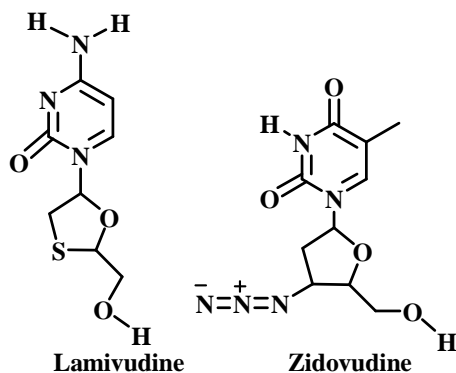
Waters HPLC (PDA Detector) empower software.

Reagents and chemicals used

Lamivudine-working standard; Zidovudine-working standard; ammonium acetate-AR grade; methanol-HPLC grade; water-Milli Q grade

Chromatographic conditions

Column : Hyper sil BDS C₁₈; 4.6150mm, 5 mi-



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crons; Flow rate : 1.5ml/min; Detection : 270nm; Injection volume : 10 μ l; Column temperature: Ambient; Run time : 15minutes.

Preparation of buffer

Dissolve 7.7grams of ammonium acetate in 1000ml of water. Filter through μ m membrane filter or fine porosity membrane filter.

Mobile phase-gradient programe

Time (min)	Buffer	Acetonitrile
0.0	93	7
2.0	93	7
8.0	75	25
9.0	75	25
10.0	93	7
15.0	93	7

Preparation of diluent

Prepare a degassed mixture of methanol and water in the ratio of 50:50v/v

Preparation of standard solution

Weigh and transfer accurately about 38mg of lamivudine and 75mg of zidovudine working standards into a 100ml volumetric flask add about 50ml of diluent and sonicate to dissolve and dilute to volume with diluent and mixwell.

Dilute 10ml of the above solution to 25ml with diluent and mix. Filter through 0.45 μ m filter.

Preparation of test solution

Weigh and powder 20 tablets. Weigh accurately a quantity of tablets powder equivalent to 300mg of zidovudine, 150mg of lamivudine separately into a 200 ml volumetric flask add about 100ml of diluent and sonicate for 20minutes with occasional stirring and dilute to volume with diluent and mix dilute 5ml of above solution to 25ml with diluent and mix and filter through 0.45 μ m filter.

Evaluation of system suitability

Inject 10 μ l of standard solution five times into the chromatographic system and record the chromatograms and measure the peak areas. The column efficiency as determined from lamivudine peak and zidovudine peak is not less than 2000 theoretical plates. The tailing factor for the same peaks are not more than 2.0. RSD for the peak areas of the five replicate injections of

lamivudine and zidovudine peaks are not more than 2.0%.

Procedure

Inject 10 μ l of sample solution into the chromatographic system and record the chromatograms and measure the peak areas. Retention time of lamivudine and zidovudine are about 2.3 and 6.5minutes respectively.

Linearity

To evaluate the linearity range of lamivudine and zidovudine, varying quantity of stock solution was diluted with mobile phase to give a minimum of seven concentrations in the range of 50%, 60%, 80%, 100%, 120%, 130% and 150%.

There is a linear response is observed with concentration in case of both drugs.

Recovery studies

Recovery studies were conducted using 50%, 100%, 150% concentrations of lamivudine and zidovudine respectively. Results and statistical parameters are reported in TABLE 1 and 2.

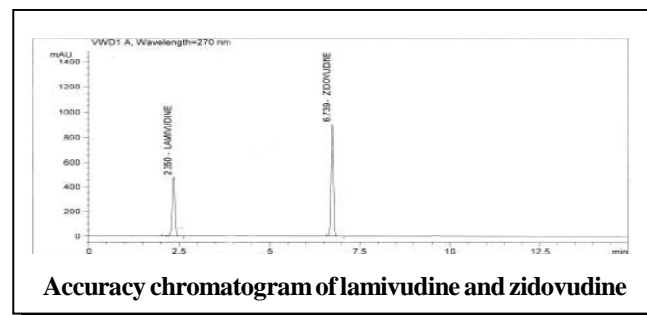


TABLE 1 : % Recovery of lamivudine

Accuracy level	Amount added in mg	Peak area	% recovered
50	75.23	1220.45	99.5
100	150.12	2410.520	98.5
150	225.16	3620.14	98.7

TABLE 2 : % Recovery of zidovudine

Accuracy level	Amount added in mg	Peak area	% recovered
50	150.19	2139.649	99.5
100	300.15	4258.716	99.1
150	450.16	6356.348	98.6

CONCLUSION**Linearity**

The plot of peak area versus the respective concentration of lamivudine and zidovudine were found to be linear in the concentration range.

Accuracy

A standard working solution containing lamivudine and zidovudine was prepared. From the respective area counts the concentrations of lamivudine and zidovudine were calculated using the detector responses. The accuracy of the proposed RP-HPTLC method was expressed in terms of recovery. The recovery studies were carried and the results expressed as percentage recovery.

Although various methods have been developed for the estimation of lamivudine and zidovudine individually and in combination with other drugs the proposed HPLC method provides simple, accurate and reproducible quantitative analysis for simultaneous determination of lamivudine and zidovudine in tablets.

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