



DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE

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ABSTRACT

A new RP-HPLC method was developed for the simultaneous estimation of lamivudine and zidovudine combination in Zidolam tablets and it was validated as per ICH guidelines.

The chromatograms for lamivudine and zidovudine were found to be satisfactory on symmetry C-18 (4.6×150mm, 5 μ Thermosil column) using mobile phase composed of 60:40%v/v phosphate buffer of pH 3.6 and methanol at a flow rate of 0.8ml/min. The HPLC system which was used consisted of a water alliance equipped with an Auto sampler and UV detector. The content of lamivudine and zidovudine in Zidolam tablets was found to be 151.57 mg and 304.06 mg per tablet respectively. The retention time of lamivudine and zidovudine was found to be 2.338 and 3.415 min respectively. The asymmetry factor or the tailing factor of lamivudine and zidovudine was found to 1.7 for each which indicates symmetrical nature of the peak. The %RSD for the intraday precision for lamivudine and zidovudine was found to be 0.10 and 0.14 respectively and %RSD for inter day precision was found to be 0.39 for Lamivudine and 0.32 for zidovudine, which confirm the validation requirement. The percent (%) recovery range was 98-99%.

The proposed RP HPLC method was found suitable for the simultaneous determination of lamivudine and zidovudine combination. The developed method for the simultaneous estimation of lamivudine and zidovudine combination in formulations is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

Keywords: RP HPLC Method, Simultaneous Estimation, Lamivudine, Zidovudine, Zidolam Tablets.

INTRODUCTION

Lamivudine¹⁻⁴ and zidovudine⁵⁻⁸, two widely prescribed anti retroviral drugs are official in IP 2010 and USP 2008. Lamivudine and zidovudine combination has significant therapeutic importance. The combination treatment is known as highly active antiretroviral therapy (HAART). Using a HAART protocol, HIV replication is inhibited, the presence of HIV-RNA in the plasma is reduced to undetectable levels and patient survival is greatly prolonged. Zidolam tablets (a commercial brand) contain lamivudine (150 mg) and zidovudine (300 mg). Zidolam tablets are used in antiretroviral combination therapy for the treatment of HIV infection. Zidolam

tablet reduces the amount of HIV in the body and keeps it at a low level. It also increases CD4 cell counts. CD4 cells are a type of white blood cells that plays an important role in maintaining a healthy immune system to fight against infection. A few methods were reported⁹⁻¹² for the simultaneous estimation of lamivudine and zidovudine combination.

Studies were carried out in the present study on lamivudine and zidovudine drug combination with an objective of developing a simple, sensitive, precise and accurate RP-HPLC method for the simultaneous estimation of lamivudine and zidovudine combination in bulk and in dosage forms and to validate the developed RP-HPLC method as per ICH guidelines.

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EXPERIMENTAL

Materials:

Lamivudine and zidovudine were gift samples from M/s Hetero Pharmaceuticals Ltd., Hyderabad. Water (HPLC grade), Methanol (HPLC Grade), Potassium dihydrogen orthophosphate (HPLC grade) and ortho phosphoric acid were procured from commercial sources. Zidolam tablets manufactured by Hetero Pharmaceuticals were procured from local market. The tablets were claimed to contain 150 mg of lamivudine and 300mg of zidovudine. All other materials used were of pharmacopoeial grade.

Methods:

The developed RP-HPLC method for the estimation of lamivudine and zidovudine combination was carried out on Thermosil Column- Symmetry C18 (150X4.6mm, id, 5 μ particle size) in isocratic mode using a mobile phase composition of phosphate buffer (pH 3.6): methanol [60:40 v/v] with a flow rate of 0.8 ml/min and detection at 276 nm.

Preparation of pH 3.6 Phosphate buffer:

2.7218g of KH_2PO_4 was weighed and transferred into a 1000ml beaker, later it was dissolved and diluted to 1000ml with HPLC water and the pH was adjusted to 3.6 with orthophosphoric acid.

Preparation of mobile phase:

A mixture of pH 3.6 Phosphate buffer 600 ml (60%) and 400 ml of HPLC grade Methanol (40%) was taken and degassed in ultrasonic water bath for 5 minutes. Later it was filtered through 0.45 μ filter under vacuum filtration. The mobile phase was used as diluents in the analysis.

Preparation of Standard Solution:

Lamivudine (10 mg) and Zidovudine (20 mg) were accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 7ml of diluent was added and sonicated to dissolve the drugs completely and the volume was made up to the mark with the same solvent. (Stock solution I). Later 5ml of solution was pipetted out from the above stock solution into a 25 ml volumetric flask and the volume was made upto the mark with the diluent (stock solution II). Further 1.5 ml of

solution was pipetted out from stock solution II into a 10ml volumetric flask and diluted to the mark with diluent (stock solution III).

Preparation of Sample Solution:

Tablet powder equivalent to 150 mg of lamivudine and 300 mg of Zidovudine i.e., 7286mg of Zidolam tablet powder was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve the drugs completely and the volume was made upto the mark with the same solvent. (Stock solution-I). Further 5 ml of solution was pipetted out from the above stock solution into a 25 ml volumetric flask and diluted up to the mark with diluent (Stock solution II). Further 1 ml of above solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent (Stock solution III).

Test Procedure:

20 μ l each of the standard and sample solutions of lamivudine and zidovudine were injected into the chromatographic system and the areas for the lamivudine and zidovudine peaks were measured and drug content of the tablets was calculated by comparing the areas of standard and sample solutions.

RESULTS AND DISCUSSION

A new RP HPLC method was developed for simultaneous determination of lamivudine and zidovudine combination. Various ratios of mobile phase systems were prepared and used and the composition containing phosphate buffer (pH-3.6) and methanol in the ratio 60:40 v/v gave a better resolution and sensitivity. The content of lamivudine and zidovudine in Zidolam tablets was found to be 151.57 mg and 304.06 mg per tablet respectively. The retention time of lamivudine and zidovudine was found to be 2.338 and 3.415 min respectively. The asymmetry factor or the tailing factor of lamivudine and zidovudine was found to 1.7 for each which indicates symmetrical nature of the peak. System suitability parameters such as theoretical plates were calculated the number of theoretical plates of lamivudine and zidovudine were found to be 2252.5 and 2830.7 respectively which indicates efficient

performance of the column. From the linearity studies, the specified concentration range was determined. The standard chromatogram for Level 5 Linearity was shown in Fig.1. It was observed that lamivudine was linear in the range of 10-50µg/ml and zidovudine was linear in the range of 20-100µg/ml. The correlation coefficient of lamivudine and zidovudine was found to be 0.999 in each case (Tables 1-2).

The validation of the proposed method was verified by precision. The %RSD for the intraday precision for lamivudine and zidovudine was found to be 0.10 and 0.14 respectively and %RSD for interday precision

was found to be 0.39 for Lamivudine and 0.32 for zidovudine, which confirm the validation requirement. The validation of the proposed method was verified by recovery studies. The percent (%) recovery range was found to be 98-99%. The Limit of Quantification and Limit of Detection were calculated from the linearity curve method using Signal to noise ratio. Limit of detection for lamivudine was found to be 0.024µg / ml and for zidovudine it was found to be 0.08 µg/ml. The limit of quantification for lamivudine and zidovudine was found to be 0.08µg/ml and 0.16 µg/ml respectively

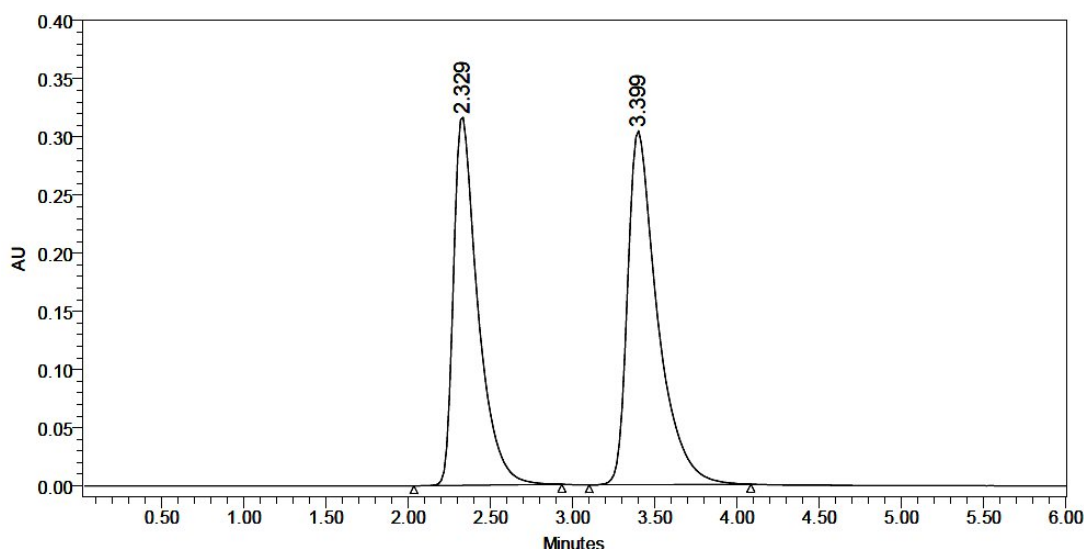


Fig 1: Standard Chromatogram for Level 5 Linearity.

Table 1: Calibration Curve of Lamivudine

S. No	Linearity Level	Concentration (mcg/ml)	Area
1	I	10	668978
2	II	20	1303018
3	III	30	1984694
4	IV	40	2611374
5	V	50	3269630
Correlation Coefficient			0.999

Table 2: Calibration Curve of Zidovudine

S. No	Linearity Level	Concentration (mcg/ml)	Area
1	I	20	784928
2	II	40	1524159
3	III	60	2329360
4	IV	80	3065982
5	V	100	3830623
Correlation Coefficient			0.999

CONCLUSIONS

1. A new RP-HPLC method was developed for the simultaneous estimation of lamivudine and zidovudine combination in Zidolam tablets and it was validated as per ICH guidelines.
2. The chromatograms for lamivudine and zidovudine were found to be satisfactory on symmetry C-18 (4.6×150mm, 5µ Thermosil column) using mobile phase composed of 60:40%v/v phosphate buffer of pH 3.6 and methanol at a flow rate of 0.8ml/min. The HPLC system which was used consisted of a water alliance equipped with an Auto sampler and UV detector.
3. The content of lamivudine and zidovudine in Zidolam tablets was found to be 151.57 mg and 304.06 mg per tablet respectively. The retention time of lamivudine and zidovudine was found to be 2.338 and

3.415 min respectively. The asymmetry factor or the tailing factor of lamivudine and zidovudine was found to 1.7 for each which indicates symmetrical nature of the peak.

4. The %RSD for the intraday precision for lamivudine and zidovudine was found to be 0.10 and 0.14 respectively and %RSD for inter day precision was found to be 0.39 for Lamivudine and 0.32 for zidovudine, which confirm the validation requirement. The percent (%) recovery range was 98-99%.
5. The developed method for the simultaneous estimation of lamivudine and zidovudine combination in formulations is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

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