



## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TELBIVUDINE IN BULK DRUGS AND PHARMACEUTICAL FORMULATIONS

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### ABSTRACT

A simple, selective, rapid and precise HPLC method was developed for the estimation of telbivudine in bulk drugs and in pharmaceutical formulations. The drug was separated on a Luna C18 (4.6×150mm, 5µm) column with mobile phase comprising of methanol: water in the ratio of 70:30% v/v. Retention time of the drug was found to be 2.8 min. Linearity of the method was found to be 10-50µg/ml. The method was validated according to the ICH guidelines. The method was found to be suitable for the routine quality control analysis of telbivudine.

### INTRODUCTION

Telbivudine (TBD) is a synthetic thymidine nucleoside analog with specific activity against the hepatitis B virus. Telbivudine is orally administered, with good tolerance, lack of toxicity and no dose-limiting side effects. The chemical name for telbivudine is

1-((2S,4R,5S)-4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-5-methyl-1H-pyrimidine-2,4-dione, or 1-(2-deoxy-β-L-ribofuranosyl)-5-methyluracil.

Telbivudine is the unmodified β-L enantiomer of the naturally occurring nucleoside, thymidine. Its molecular formula is C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub>, which corresponds to a molecular weight of 242.23. Structure of telbivudine is given in figure 1.

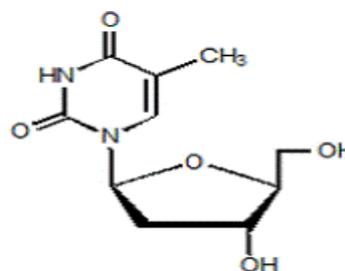


Figure 1: Structure of telbivudine

Telbivudine is a white to slightly yellowish powder. Telbivudine is sparingly soluble in water (greater than 20 mg per mL), and very slightly soluble in absolute ethanol (0.7 mg per mL) and n-octanol (0.1 mg per mL). Telbivudine inhibits HBV DNA

polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5'-triphosphate. This leads to the chain termination of DNA synthesis, thereby inhibiting viral replication. Incorporation of telbivudine into viral DNA also causes DNA chain termination, resulting in inhibition of HBV replication. Telbivudine inhibits anticomplement or second-strand DNA. Literature survey revealed that few HPLC [1-2] and LC-MS/MS [3-8] methods have been reported for the estimation of telbivudine in bulk drugs, pharmaceutical formulations and biological fluids. In the present investigation we have developed simple and sensitive HPLC method for the estimation of telbivudine in bulk drugs and pharmaceutical formulations.

## EXPERIMENTAL:

### Chemicals, Reagents, and Solutions:

Pharmaceutical grade of Telbivudine was kindly supplied by Sura Labs (Hyderabad, India). Water, methanol and acetonitrile (HPLC grade) were purchased from Merck, India. Standard stock solution of TBD was prepared in methanol at a concentration of 1.0 mg/mL and further diluted with methanol to furnish working standard stock solution of 100 µg/mL. The working standard stock solution was used to prepare calibration samples. Mobile phase was prepared by mixing 300 ml of HPLC Water with 700 ml of Methanol and degassed by sonication for 10 minutes and then filtered through 0.45 µ membrane filter. Mobile phase was also used as diluent.

### HPLC Instrumentation and Chromatographic Conditions:

HPLC system used for the investigation was Shimadzu HPLC with manual injector and PDA Detector. Software used was the Labsolution and column used was Luna C18 (4.6×150mm, 5 µ). Mobile phase used was methanol: water in the ratio of 70:30 v/v and flow rate was 1 mL/min. Run time used for the chromatography was 6 min. and analyte was monitored at 270 nm.

### Standard Solutions and Calibration Curve:

The standard stock solution was diluted with mobile phase to prepare working standard solution and calibration samples. Calibration

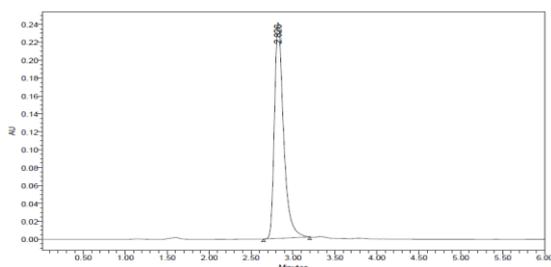
samples were prepared in the concentration range of 10 to 50 µg/mL. Triplicate injections were made for each calibration sample and chromatographed under the specified HPLC conditions described previously. The peak area of each concentration was plotted against the corresponding concentrations to obtain the calibration graph. Linear relationship was obtained.

### Analysis of the Pharmaceutical Dosage Forms:

Tablet powder equivalent to 10 mg of TBD was taken in 10 mL volumetric flask, and volume was made up with diluent, vortexed for 10 min, and sonicated for 15 min. Further pipette out 0.3ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. The resultant solution was injected into the HPLC system and amount of drug present in the formulation was calculated.

## RESULTS AND DISCUSSION:

HPLC method was developed by performing different runs with different mobile phase combinations like acetonitrile: water; methanol:TEA buffer; methanol:water:acetonitrile; phosphate buffer: acetonitrile and methanol: water at different proportions and different columns. The peak shape was good by using the mobile phase methanol: water in the ratio of 70:30. The column used was C18 column. The optimized chromatogram was shown in figure 2. The retention time of the drug was found to be 2.8 min. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines [9], for system suitability, linearity, specificity, precision, accuracy, limit of detection, limit of quantification, and robustness. System-suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. % RSD of retention time, number of theoretical plates ( $N$ ), and tailing factor ( $T$ ) were evaluated for five replicate injections of the drug at a concentration of 30 µg/mL. The % RSD of retention time was found to be  $\leq$  2%, number of theoretical plates was found to be 3970 and tailing factor observed was 1.4.



**Figure 2:** The optimized chromatogram of TBD at a concentration of 30 µg/ml.

The method was found to be specific as there is no interference from the excipients present in the formulation. Linearity of the proposed method was evaluated according to ICH guidelines. TBD showed linearity in the concentration range of 10-50 µg/mL. The regression equation obtained was  $Y = 24835X + 22967$ , where Y is peak area and X is concentration of TBD (µg/mL). The correlation coefficient was found to be 0.998. Precision of the method was checked by repeatability and intermediate precision. Repeatability of the method was checked by five replicate injections of 100% accuracy solution. The % RSD of the peak area was found to be 0.17, within the acceptable limit of not more than 2. Intermediate precision was checked by injecting the 100% accuracy solution for six times on different days. %RSD of the peak area was found to be 0.2, within the acceptable limit of not more than 2. Accuracy of the method was performed at 50, 100 and 150% concentration level and percentage recovery was within the acceptable limit of 98-102%. Limit of detection (LOD) is the lowest concentration of drug that can be detected but not necessarily quantifiable and limit of quantification (LOQ) was the lowest amount of drug that can be quantifiable with suitable accuracy and precision. LOD and LOQ values were found to be 3 and 9 µg/ml, respectively. A method is robust if it is unaffected by small changes in operating conditions. To evaluate HPLC method robustness, a few parameters were deliberately varied. The parameters included variation of flow rate and variation of mobile phase composition. Flow rate varied was  $\pm 0.1$  ml and content of mobile phase was varied by  $\pm 10\%$ . The method was found to be robust. The assay of the tablets was found to be 100.8%.

## CONCLUSION:

A simple, selective, rapid and precise HPLC method was developed for the estimation of telbivudine in bulk drugs and in pharmaceutical formulations. The method was validated in terms of system suitability, specificity, linearity, accuracy, precision, LOD&LOQ and robustness. The method was found to be suitable for the routine quality control analysis of telbivudine.

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