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Estimation of Entecavir in Tablet Dosage Form by RP-HPLC

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A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of entecavir in tablet dosage form. A XTerra(R) C18, 250 mm × 4.6 mm, 5 µm particle size, with mobile phase consisting of water:acetonitrile in the ratio of 80:20 (v/v) was used. The flow rate was 0.8 mL/min and the effluents were monitored at 254 nm. The retention time was 3.385 min. The detector response was linear in the concentration of 2-24 mcg/mL. The respective linear regression equation being Y= 10843.972x + 7662.8569. The limit of detection and limit of quantification was 0.1mcg and 0.3 mcg/mL, respectively. The percentage assay of entecavir was 99.07 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of entecavir in bulk drug and in its pharmaceutical dosage form.

Key Words: Entecavir, RP-HPLC.

INTRODUCTION

Entecavir[1] is a novel nucleoside analogue reverse transctipase inhibitor drug that has selective anti haptitis B virus (HBV) activity. It is a deoxy guanine nucleoside analogue, inhibits hepatitis B-virus (HBV) DNA polymerase[2]. Chemically, it is 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-hydroxymethyl)-2-methylenecyclopentyl]-6*H*-purine-6-one[3]. Its molecular weight is 277.28 and molecular formula is $C_{12}H_{15}N_5O_3$. Literature survey reveals no chromatographic methods for the estimation of entecavir from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of entecavir in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of entecavir in bulk drug samples and in pharmaceutical dosage form.

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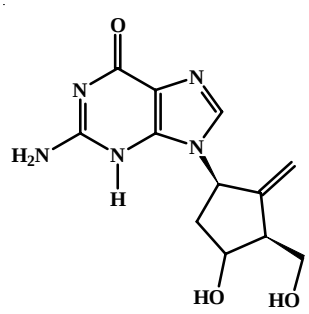

Structure of entecavir

EXPERIMENTAL

Entecavir was obtained as a gift sample from Hetero Drugs Ltd, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available entecavir tablets (Baraclude 1 mg, Bristol-Myers Sqibb) were procured from local market.

Quantitative HPLC was performed on liquid chromatograph, waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µL and 2693 pump. A RP C-18 XTerra(R) column (250 mm × 4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions: The contents of the mobile phase were water and acetonitrile in the ratio of 80:20 (v/v). They were filtered before use through a 0.45 µm membrane filter and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 mL/min. The run time was set at 10.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the system. The eluents were monitored at 254 nm.

Preparation of standard stock solution: A standard stock solution of the drug was prepared by dissolving 20 mg of entecavir in 100 mL volumetric flask containing 30 mL of water, sonicated for about 15 min and then made up to 100 mL with water to get 200 mcg/mL standard stock solution.

Working standard solution: 5 mL of the above stock solution was taken in 50 mL volumetric flask and there after made up to 50 mL with mobile phase to get a concentration of 20 µg/mL.

Preparation of sample solution: Twenty tablets (Baraclude 1mg, Bristol-Myers Sqibb) were weighed and then powdered. A sample of the powdered tablets, equivalent to 1 mg of the active ingredient, was mixed with 30 mL of water in 50 mL volumetric flask. The mixture was allowed to stand for 1 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 µm membrane filter, followed by adding water up 50 mL to obtain a stock solution of 20 mcg/mL.

Linearity: Aliquots of standard entecavir stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of entecavir are in the range of 2-24 µg/mL. Each of these drug solutions (20 µL) was injected three times into the column and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 254 nm.

The plot of peak area of each sample against respective concentration of entecavir was found to be linear in the range of 2-24 µg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective linear regression equation being Y = 10843.972x + 7662.8569. The regression characteristics, such as slope, intercept and % RSD were calculated for this method and given in Table-1.

TABLE-1
LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Drug	Entecavir
Concentration range (µg/mL)	2-24
Slope (m)	10843.972
Intercept (b)	7662.8563
Correlation coefficient	0.9999
% RSD	0.2200
Standard error of estimate	5616.946

Assay: 20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3.385 min. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table-2.

TABLE-2
RESULTS OF HPLC ASSAY AND RECOVERY STUDIES

Sample	Amount claim (mg/tablet)	% Found by the proposed method	Recovery* (%)
1	1	99.85	101.37
2	1	100.52	99.92
3	1	99.26	99.37

*Average of three different concentration levels.

Recovery studies: Accuracy was determined by recovery studies of entecavir, known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table-2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of entecavir. Parameters that were studied to evaluate the suitability of the system are given in Table-3.

TABLE-3
VALIDATION SUMMARY

Validation parameter	Results
Theoretical plates (N)	7926.820
Tailing factor	1.180
Retention time (min)	3.385
Resolution	5.090
Area (%)	99.880
LOD (µg/mL)	0.01
LOQ (µg/mL)	0.03

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) for entecavir were found to be 0.01 and 0.03 µg/mL, respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

The typical chromatogram of entecavir showed the retention time is 3.385 min. A mixture of water and acetonitrile in the ratio of 80:20 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r = 0.9999) was observed between the concentration range of 2-24 µg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of entecavir tablets was found to be 99.07 %. From the recovery studies it was found that about 99.17 % of entecavir was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of entecavir within a short analysis time.

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