A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIATION OF ENTECAVIR IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for the estimation of entecavir in bulk and pharmaceutical dosage forms. The method is based on the reaction of entecavir with 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) to form a red colored charge–transfer complex. The red colored solution is measured at 461 nm against reagent blank. Results obtained are statistically validated.

Key words: Spectrophotometry, 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone, entecavir, Pharmaceutical and Formulation

MATERIALS AND METHOD

Instrument:

All measurement were done on Milton Roy 1001spectrophotometer by using 10 mm matched quartz cuvettes.

Preparation of reagents and solutions:

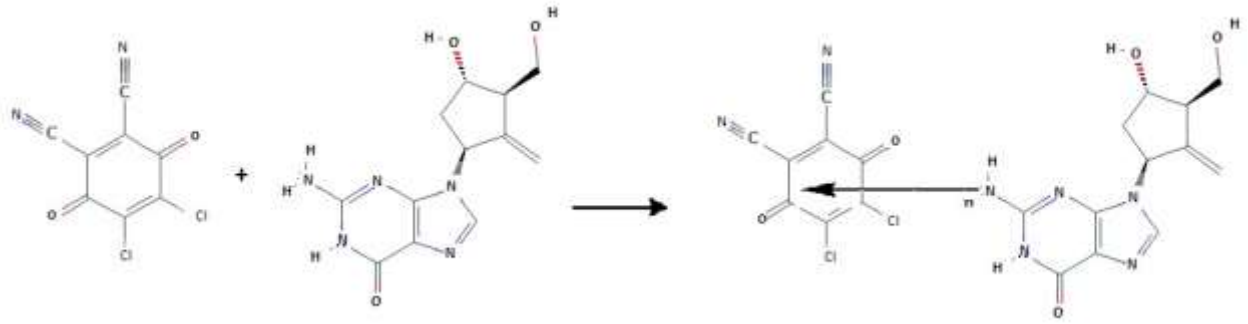
DDQ (100µg/ml):

DDQ (2,3-dichloro 5,6-dicyano-p-benzoquinone) (Loba Chem., India) is prepared by dissolving 80 mg of DDQ in 100 ml of methanol and further diluted this solution to obtained 100 µg/ml.

INTRODUCTION

Entecavir chemically designation as 2-amino-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl]-1H-purin-6-one. Entecavir 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. The method is based on the reaction of entecavir with 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone (DDQ) to form an orange red colour charge–transfer complex. The orange red colour solution is used to determine the entecavir spectrophotometrically. Various analytical methods have been reported in literature which includes, spectrophotometric method[1-5], RP-HPLC method[6], HPLC method[7,8].

The reaction sequence of the drug treated with DDQ is as follows in scheme.1



Scheme 1: Reaction sequence of entecavir treated with DDQ

Assay Procedure:

Various aliquots of the standard entecavir solution ranging from 0.2-1.0 ml are transferred into a series of standard flasks. To each flask, 1.0 ml of DDQ solution is added to produce an orange red colour. The final volume is brought to 10 ml with methanol. The reaction mixture in each flask is well shaken and allowed to stand for 5 min to complete the reaction. The absorbance of the orange red colour solution is measured at 461 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of entecavir solution. The calibration curve is found to be linear over a concentration range of 20 to 100 μg/mL of entecavir. The amount of entecavir present in the sample is read from the calibration graph. The results are presented in fig.1.

Assay of entecavir in bulk samples:

50 mg of pure entecavir is dissolved in methanol and the volume is made upto 50 ml with methanol. Further dilution is made as described in the preparation of standard solution of entecavir. Further analysis is carried out as per procedure described above and results are summarized in the Table.1. The amount of drug present in the sample is estimated from calibration graph.

Results and discussion:

In this method the drug react with DDQ solution to form an orange red charge complex. The orange red colored charge complex solution formed is measured at 461 nm against reagent blank. The amount of drug read from calibration curve. The calibration curve is linear over the range of 20-100 μg/mL of entecavir. The optical

characteristics of the proposed method such as absorption maxima, Beer´s law limits, molar absorptivity and Sandell´s sensitivity are presented in Table .1. The molar absorptivity and Sandell´s sensitivity values show sensitivity of the method. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the Table .1. The value of correlation coefficient (r) was 0.999, which indicated the good linearity of calibration lines.

Table.1. Optical characteristics of the proposed methods

parameters	Proposed method
λmax (nm)	461
Beer's law limit (µg/ml)	20-100
Molar absorptivity (l mole-^{1} cm-^{1})	5.169x10^{3}
Sandell's sensitivity (µg cm-^{2} / 0.001 absorbance unit)	0.05712
Regression equation (Y = a + bx)	Y=0.0044X+0.0036
Slope (b)	0.0044
Intercept (a)	0.0036
correlation coefficient (r)	0.9995

*Y = a+bx, where Y is the absorbance and X concentration in 50 µg/ml

Table.2. Assay of entecavir in tablet formulations

Tablets	**Labeled amount(mg)**	***Amount found (mg)±S.D***	**% label claim**	***t value**
Tablet 1	1	1.01±0.33	101.0	0.1070

*Average of five determination based on label claim

CONCLUSSION

The percent relative standard deviation calculated from the five measurements of entecavir shown in Table .2. The % RSD is less than 2, which indicates that the method has good reproducibility. The values of standard deviation

values are low, indicates high accuracy and reproducibility of the method. The 't' calculated values are compares well with the theoretical value of 2.78 there by indicating that the precision of the method.

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of entecavir in bulk drugs samples.

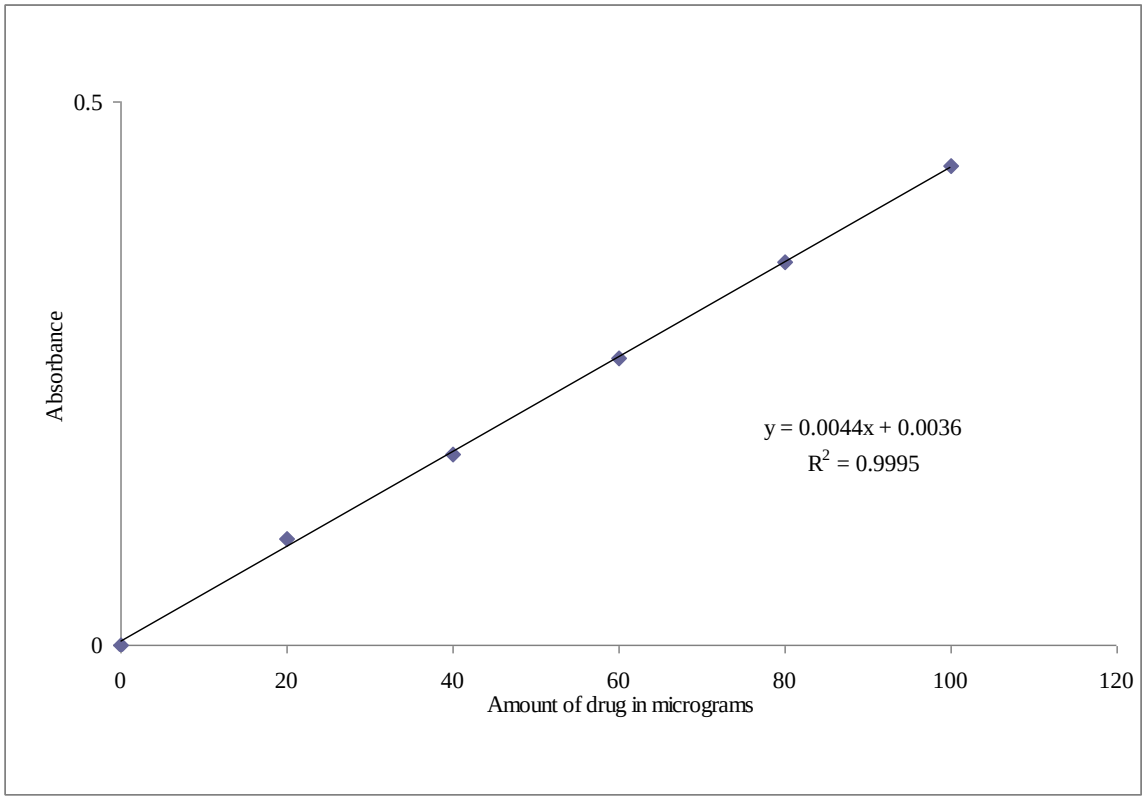


Fig.1: Calibration curve of entecavir

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