Biomedical & Pharmacology Journal Vol. **1**(2), 417-420 (2008)

Spectrophotometric estimation of entecavir in pharmaceutical formulations

V. KIRAN KUMAR[1] and N.APPALA RAJU*

[1]Department of Phamacognosy, Vaageswari College of Pharmacy, Karimnagar (India).
*Department of Pharmaceutical Chemistry, Sultan-Ul-Uloom College of Pharmacy, Mount Pleasant, Road No.3, Banjara Hills, Hyderabad - 500 034 (India).

(Received: August 25, 2008; Accepted: October 12, 2008)

ABSTRACT

Three simple accurate, rapid and sensitive methods have been developed for the estimation of Entecavir in the pharmaceutical dosage form. The Ninhydrin Method is based on reaction of Entecavir with Ninhydrin to form a colored compound. The Ascorbic acid method is based on reaction of Lamiudine with Ascorbic acid to form a purple colored compound. The PBQ Method is based on the reaction of Entecavir with carbonyl group of PBQ to form the condensation product. These Methods exhibits maximum absorption at 580nm, 535nm, 395nm respectively and obeys the Beer's law in the concentration range of 5-60mcg/ml, 5-80mcg/ml, and 10-60mcg/ml respectively. The Methods have been statistically evaluated and were found to be precise and accurate. The proposed Methods are economical and sensitive for the estimation of Entecavir in the bulk drug and in its formulations.

Key words: UV-Visible Spectrophotometry, Entecavir, Ninhydrin, Ascorbic acid, *p*- Benzoquinone (PBQ).

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-purine6-one[1]. Its molecular weight is 277.28 and molecular formula is $C_{12}H_{15}N_5O_3$. Literature survey reveals no spectrophotometric methods and chromatographic methods have been reported for the estimation of Entecavir from pharmaceutical dosage forms. The availability of visible spectrophotometric methods with high sensitivity and selectivity will be very useful for the determination of Entecavir in pharmaceutical formulations. The present study describes simple, sensitive, accurate, rapid and economical Spectrophotometric Ninhydrine Methods, Ascorbic acid Method, and PBQ Method for the estimation of Entecavir in its formulations.

EXPERIMENTAL

Instument

Elico Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

Materials and reagents

All chemicals used were of analytical reagents grade. Entecavir was obtained from Hetero Drugs, Hyderabad.

1. Ninhydrin (2%w/v): The 2% Ninhydrin solution was prepared in *N, N'* –dimethylformamide (DMF).
2. Ascorbic acid (1%w/v): Ascorbic acid solution was prepared by dissolving 1000mg in 10mL of distilled water, in a 100mL volumetric flask and completing the volume with DMF.

3. *p*-Benzoquinone (PBQ) (0.5%w/v): PBQ solution was prepared in methanol. 0.1M phosphate ($Na\ H_2PO_4$) buffer solution was prepared and pH adjusted to 7.5 with NaOH.

Standard solution

Stock solution (1000mcg/ml) was freshly prepared by dissolving 10mg of Entecavir in 10mL of distilled water and then this was further diluted water so as to obtain working standard solutions of 100mcg/ ml for all the three propose methods.

Preparation of the sample solution

20 tablets (Baraclude 1mg, Bristol-Myers Sqibb) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 1mg of the active ingredient, was mixed with 5 ml of water in 10 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, followed by adding water up 10 ml to obtain a stock solution of 100mcg/ ml.

General procedures

Ninhydrin method

In to 10mL volumetric flasks, different aliquots of working standard solution (0.5-6mL) were transferred to provide final concentration range 50-600mcg/mL. To each flask, 2mL of 2%w/v Ninhydrin solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 10 min. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was measured at 575nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the Entecavir. The content of the unknown was computed either from calibration curve.

Ascorbic acid method

In 10mL volumetric flasks, different aliquots of working standard solution (0.5- 8 ml) were transferred to provide final concentration range 50-800 mcg/ml. To each flask, 1.5ml of 1% ascorbic acid solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 15 minutes. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was measured at 530 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the Entecavir. The content of the unknown was computed from calibration curve.

PBQ method

In to 10mL volumetric flasks, different aliquots of working standard solution (1-6 ml) were transferred to provide final concentration range 100 -600mcg/ml. To each flask, 1.5 ml of 0.1 M phosphate buffer solution and 1.5 ml of PBQ reagent were successively added. The volume was made up to mark with distilled water and the solutions were heated on a boiling water bath 10 minutes. The solutions were cooled to room temperature and made up to mark with distilled water. The absorbance of each solution was measured at 400 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of Entecavir. The content of the unknown was computed from calibration curve.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen the effect of quantity, concentration and order of addition of various reagents were studied, optimized after several experiments and incorporated in the procedure.

Ninhydrin method

That Ninhydrin was converted to *o*-Carboxyphenylglyoxal in alkaline medium, which would reduce Ninhydrin to 2-hydroxyindan-1, 3-Dione. In the present study, it combines with $-NH_2$ group of Entecavir to from amino derivative, which further undergoes condensation with Ninhydrin to give diketohydrindylindene-diketohydrindamine (Ruhemenn's purple) with maximum absorption at 575nm. The reaction between Entecavir and Ninhydrin in DMF resulted in the formation of diketohydrindylindene-diketohydrindamine. Entecavir was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating on a boiling water bath for 10 minutes. The developed color was stable for 2hrs.

Table 1: **Optical characteristics and precision data**

Parameters Method	**Nihydrine Method**	**Ascorbic acid Method**	**PBQ**
λ_{max} (nm)	580	535	395
Beer's law limits mcg/ml	5-60	5-80	10-60
Molar absorptivity (l/mol.cm)	$3.59x10^3$	$6.4 x10^3$	$4.36 x10^3$
Sandell's sensitivity(mcg/cm^2/0.001absorbance unit)	0.093	0.0792	0.0645
Regression Equation* (Y)Slope (m)	0.009	0.013	0.014
Intercept (c)	0.0807	-0.0113	0.0213
Correlation Coefficient(r)	0.992	0.997	0.987
Precision (%Relative Standard Deviation)	0.1025	0.2625	0.0787
Standard error of mean	0.0374	0.0229	0.0487
Confidence Intervels 99	0.0208	0.2426	0.0621
95	0.0205	0.0107	0.0596

*Y=mx+c, where X is the concentration in micrograms/ml and Y is absorbance unit.

Table 2: Assay and recovery of entecavir in tablet dosage form

Tablet formulation	**Labelled Amount (mg)**	**Amount Obtained (mg)* by proposed method**			**** % Recovery by the Proposed method**		
		Method A.	**Method B**	**Method C**	**Method A**	**Method B**	**Method C**
1	1	0.96	1.05	0.96	99.5	99.9	98.7
2	1	0.95	1.03	1.02	99.4	101.2	99.2
3	1	1.03	0.98	1.01	99.9	100.1	99.4

Ascorbic acid method

Entecavir, as a primary amine, reacts with ascorbic acid in DMF medium to produce a colored product, which absorbed maximally at 530 nm. Under the specified experimental conditions, ascorbic acid undergoes oxidation resulting in the formation of dehydroascorbic acid. The carbonyl group of dehydroascorbate reacts with $-NH_2$ group of Entecavir to form a purple colored condensation product.

***p*- Benzoquinone (PBQ) method**

The free primary amine moiety of Entecavir condenses with carbonyl group of PBQ to form the condensation product.

The optical characteristic such as absorption maxima, beer's law limits, molar absorptivity and sandell's sensitivity, regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation results are summarized in Table 1. The results are represented in Table 2. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Entecavir, by the proposed methods.

In conclusion, the proposed methods are economical, simple, sensitive and accurate for the routine estimation of Entecavir in bulk as well as tablet form.

ACKNOWLEDGEMENTS

The authors are grateful to M/S. Hetero Drugs for the supply of Entecavir as gift sample and to the Management, Vaageswary College of pharmacy, Karimnagar, for providing the necessary facilities to carry out the research work.

REFERENCES

1. The Merck Index, **14**: 613 (2006).
2. Martindale-The Complete Drug Reference, **34**: 366 (2005).
3. P.Honkoop, R.A.de Man, Review of pharmacology and clinical experience, *Expert Opin. Invest. Drugs,* **12**: 853-871 (2004).