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RESEARCH ARTICLE

PHARMACEUTICAL ANALYSIS

NOVEL SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ENTECAVIR IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Three simple and sensitive Spectrophotometric methods (A, B & C) have been developed for the estimation of Entecavir (ETV) in pure and pharmaceutical dosage forms. Method A is based on the formation of colored species by redox reaction with Folin-Ciocalteau (FC) phenol's reagent under alkaline conditions (λmax: 750 nm). Method B is based on oxidative coupling of the drug with 3-methyl-2-benzothiazolinone hydrazone HCL (MBTH) in the presence of ferric chloride (λmax: 650 nm).Method c is based on formation of Schiff's base by the condensation of drug with p-dimethyl amino benzaldehyde (PDAB) under acidic conditions (λmax: 430 nm). These methods have been statistically evaluated and found to be precise and accurate.

KEY WORDS

Entecavir and Spectrophotometry.

INTRODUCTION

Entecavir[1-2] (ETV) is chemically 2-amino-1, 9-dihydro-9-[(1S, 3r, 4s)-4-hydroxy-3-(hydroxymethyl)-2-methylene cyclopentyl)-6H purine-6-one monohydrate, a guanosine nucleoside derivative with selective clinical activity against hepatitis B virus. A number of methods such as HPLC[3] and LCMS[4] were reported for the estimation of ETV. Literature survey reveals that a few visible Spectrophotometric methods have been reported for its quantitative determination in its pure form and pharmaceutical formulations. In the present investigation, three simple and sensitive Spectrophotometric methods have been developed for the determination of ETV. The developed methods involve the formation of colored complexes based on aromatic amino group present in the drug. In method A, FC[5-8] reagent reacts with Entecavir under alkaline conditions to form a blue colored species. Huning and Fritsch described oxidative coupling of MBTH[9-15] with amines in presence of an oxidant. Method B utilizes this reaction for the estimation of Entecavir to form a colored chromogen. Method C is based on formation of Schiff's base by the condensation of drug with PDAB[16-20]. Beer's law is obeyed and results of analysis for the three methods have been validated statistically and by recovery studies.

MATERIALS AND METHODS

Instrument:

A Systronics Model 2201 UV-VIS spectrophotometer was used for the measurements.

Reagents:

All the chemicals used were of analytical grade. The commercially available FC reagent (2 N) was taken and diluted suitably with distilled water. Methanolic solution of PDAB (0.5% w/v), MBTH (0.2% w/v), Sodium carbonate (20% w/v) and Ferric Chloride (0.1% w/v) were prepared.

Standard drug solution:

The stock solution of Entecavir was prepared by dissolving 1mg of drug in 25 ml of distilled water to get 40 µg/ml required working standard solution.

Sample solution:

Twenty tablets of ETV were weighed and powdered. A quantity of powder equivalent to 1mg was dissolved in 25 ml of distilled water. The solution was sonicated for 15 min, filtered and made up to the mark with water.

Assay Procedures

Method A

Aliquots of standard solution of Entecavir ranging from 0.5 -2.5 ml were transferred into a series of 10 ml volumetric flasks. To these, 1 ml of FC reagent and 1ml of Sodium carbonate were added and the volume was adjusted to 10mL with distilled water. The blue colored chromogen, thus formed, was measured at 750 nm against reagent blank. The amount of ETV was computed from its calibration plot.

Method B

Aliquots of working standard solution of Entecavir ranging from 0.5-2.5 ml were transferred into a series of 10 ml volumetric flasks. To these, 1.5ml of MBTH and 2ml of ferric chloride were added and kept aside for 15 min. The final volume was adjusted to 10ml of distilled water. The absorbance of the colored chromogen was measured at 650 nm against reagent blank.

The amount of Entecavir present in the sample solution was computed from its calibration curve.

Method C

Aliquots of working standard solution of Entecavir ranging from 0.25-1.25 ml were transferred into a series of 10 ml volumetric flasks. To these 2ml of PDAB and 3ml of Conc. H_2SO_4 were added, and the volume was equalized in all flasks using methanol. The contents were heated at about 60 ^oC -70^oC for 20 min and then cooled. The total volume was made up to 10ml with methanol. The absorbance of the colored chromogen was measured at 430 nm against reagent blank. The amount of drug present in the sample solution was computed from its calibration curve.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation and percent range of error (0.05 and 0.01 confidence limits) were calculated for all the three methods. The results are summarized in Table 1.The precision and accuracy were performed by analyzing six replicate samples containing known amount of drug and the results were summarized in Table1. The values obtained for the determination of ETV in pharmaceutical formulations (tablets) by the proposed methods are presented in Table 2. Studies reveal that the common excipients and other additives usually present in the tablets did not interfere in the proposed methods.

RESULTS AND DISCUSSION

Table-1

Optical characteristics, precision and accuracy of the proposed method

Parameters	Method A	Method B	Method c
λ_{max} (nm)	750	650	430
Beer's law limit(µg/mL)	8-80	6-60	1-10
Sandell's sensitivity(µg/cm^2/0.001 abs. unit)	0.07018	0.00905	0.0282
Molar absorptive(litre.mole^{-1}.cm^{-1})	5.578x10^3	4.1887x10^4	1.389x10^4
Regression equation (Y*)			
Slope(b)	0.0307	0.1234	0.0987
Intercept(a)	0.00033	0.00045	0.00049
Correlation coefficient(r)	0.9998	0.9999	0.9996
%Relative standard deviation**	0.456	0.987	0.975
%Range of error			
0.05 significance level	0.593	0.856	0.865
0.01 significance level	0.938	1.342	0.987

Average of Six Determinations

****Average of Three Determinations***

Table-2

Estimation of Entecavir in Pharmaceutical dosage Forms

Formulations	Labeled amount (mg)	Amount found* by proposed method			% recovery** by proposed method		
		Method A	Method B	Method C	Method A	Method B	Method C
Tablet 1	0.5	0.496	0.498	0.495	99.98	99.97	99.95
Tablet 2	1	0.998	0.999	1.009	101.25	100.50	99.99

* Average of six determinations

**Recovery of amount added to the pharmaceutical formulation (Average of three determinations)

CONCLUSION

The proposed methods are applicable for the assay of ETV and have an advantage of wider range under Beer's law limits. The proposed methods are simple, selective and reproducible and can be used in the routine determination of ETV in pure form and formulations with reasonable precision and accuracy.

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