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RESEARCH ARTICLE

PHARMACEUTICAL ANALYSIS

DEVELOPMENT OF NEW VISIBLE 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 determination Entecavir (ETV) in pure and pharmaceutical dosage forms. Method A is based on the formation of colored species by nucleophilic substitution of the drug with 1, 2-Napthoquinone-4-sulfonate sodium (NQS) in the presence of alkaline medium (λ_{max} :450 nm). Method B is based on formation Schiff's base by the condensation of drug with p-dimethyl amino cinnamaldehyde (PDAC) under acidic conditions (λ_{max} :520 nm). Method C is based on oxidation followed by complex formation with 1, 10-Phenanthroline (PTL) in the presence of ferric chloride (λ_{max} :500 nm).These methods have been statistically evaluated and found to be precise and accurate.

KEY WORDS

Entecavir, Spectrophotometry.

INTRODUCTION

Entecavir (ETV)[1] 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[2] and LCMS[3] were reported for the estimation of ETV. Literature survey reveals that 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, NQS[4] reacts with Entecavir under alkaline conditions to form orange colored species. Method B is based on formation of Schiff's base by the condensation of drug with PDAC[5,8]. Method C is based on oxidation followed by complex formation with 1, 10-Phenanthroline[9,12] (PTL) in the presence of ferric chloride to form a colored chromogen. 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. NQS (0.05%), Sodium hydroxide (0.5 N), Methanolic solution of PDAC (0.5%w/v), 1, 10-Phenanthroline (0.2%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 25ml of distilled water (except for method B, in which methanol is used) 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 25ml of distilled water (except for method B in which methanol is used). The solution was sonicated for 15min, filtered and made up to the mark with water.

ASSAY PROCEDURES

Method A

Aliquots of standard solution of ETV ranging from 0.15 -0.75 ml were transferred into a series of 10 ml volumetric flasks. To these, 3.5 ml of NQS reagent and 1ml of NaOH were added and kept aside for 15 min. And then the volume was adjusted to 10ml with distilled water. The colored chromogen thus formed was measured at 450nm against reagent blank. The amount of ETV was computed from its calibration plot.

Method B

Aliquots of working standard solution of ETV ranging from 0.3-1.5 ml were transferred into a series of 10 ml volumetric flasks. To These 1ml of PDAC and 2 ml of Conc. H_2SO_4 were added, the volume was equalised in all flasks using methanol. The contents were heated for 20 min and then

cooled. The total volume was made up to 10 ml with methanol. The absorbance of the colored chromogen was measured at 520nm against reagent blank. The amount of drug present in the sample solution was computed from its calibration curve.

Method C

Aliquots of working standard solution of ETV ranging from 0.5-2.5 mL were transferred into a series of 10 mL volumetric flasks. To these, 1mL of ferric chloride and then 1ml of 1, 10-Phenanthroline was added. The volume was equalised with water and kept for boiling for 15 min. The flasks were cooled to room temperature and 2ml of O-phosphoric acid was added to each flask, finally the volume was brought to 10 ml with distilled water. The absorbance was measured at 500nm against reagent blank. The amount of Entecavir present in the sample solution was computed from its calibration curve.

RESULTS AND DISCUSSION

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 summarised 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.

Table-1

Optical characteristics, Precision and Accuracy of the proposed methods

Parameter	Method A	Method B	Method c
λ_{max} (nm)	450	520	500
Beer's law limit(µg/mL)	0.6-3.0	1.0-10.0	2.0-50
Sandell's sensitivity(µg/cm^2/0.001 abs. unit)	0.00217	0.00674	0.05917
Molar absorptive(litre.mole^{-1}.cm^{-1})	1.358X10^5	4.380X10^4	0.499X10^4
Regression equation (Y*)			
Slope(b)	0.015	0.003	0.001
Intercept(a)	0.195	0.138	0.012
Correlation coefficient(r)	0.996	0.999	0.997
%Relative standard deviation**	0.2723	0.6795	0.5635
%Range of error			
0.05 significance level	0.2276	0.5681	0.4711
0.01 significance level	0.3368	0.8406 0.6970	

**Y=a+bX, where Y is the Absorbance and X is the Concentration*

*** For Six Replicates*

Table-2

Estimation of Entecavir in Pharmaceutical dosage Forms

Formulation	Labelled amount (mg/ tablet)	Amount found* by proposed methods			% recovery** by proposed methods		
		Method A	Method B	Method C	Method A	Method B	Method C
Tablet 1	0.5	0.49	0.48	0.51	99.90	99.98	99.99
Tablet 2	1	1.07	1.09	1.02	100.2	100.4	99.89

** 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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