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**Original Article**

# Estimation and validation of entecavir in bulk and pharmaceutical dosage forms by UV spectrophotometry

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## ABSTRACT

A simple, sensitive and economical UV-Spectrophotometric method was developed for the estimation of Entecavir in bulk and pharmaceutical dosage form. The absorption spectra in the proposed method, $\lambda_{max}$ of Entecavir was found to be 254nm. The calibration curve was linear over the concentration range of 3-18µg/ml with correlation coefficient of 0.999. A Percentage recovery of Entecavir ranges from 99.53-100.41%w/w indicating that the developed method is accurate and % RSD was less than 2 which indicates a good accuracy of the method. The limit of detection and limit of quantization were found to be 1.25µg/ml &3.81µg/ml. The proposed method will be suitable for the analysis of Entecavir in bulk and pharmaceutical formulation.

**Key words:** Entecavir, Relative standard deviation, $\lambda_{max,}$ Spectroscopy, limit of detection

## INTRODUCTION

Entecavir [1-3] is an anti-viral drug used for the treatment of hepatitis B. It has potent and selective activity against HBV with few side effects. Entecavir, 2-amino-1, 9-dihydro-9-[(1S, 3R, 4S)-4-hydroxy-3-(hydroxy methyl)-2- Methylene cyclopentyl) ]-6H-purine-6-one. Entecavir is a nucleoside analog that inhibits reverse transcription, DNA replication and transcription in the viral replication process. Entecavir is more efficacious than previous agents used to treat hepatitis B (lamivudine and adefovir). The drug was approved by the US FDA in March 2005. Entecavir is also indicated for the treatment of chronic hepatitis B in adults with HIV/AIDS infection. Literature survey reveals that only few analytical methods such Visible [4-8], RP-HPLC [9-13], IR and UV [15-18] methods have been reported for the estimation of Entecavir in bulk and pharmaceutical dosage form. Hence, on the basis of literature survey, it was thought to develop a precise, accurate, simple, specific and reliable method for the estimation of Entecavir.

## MATERIALS AND METHOD

***Instrument & Materials:*** Elico SL 218 and Elico SL 210 double beam UV-VIS spectrophotometers, with wide range photodiode detection and fixed 10mm path holders for reference and sample. Entecavir was obtained as gift samples from Mylan Laboratories Pvt. Ltd, Hyderabad. Methanol, distilled water of AR grade, procured from Rankem Chemicals Ltd., Mumbai.

***Method Development:***

***Preparation of Standard Stock Solution:*** Methanol and water in 1:1 ratio has been selected as solvent. 25mg of pure drug was weighed accurately and transferred into a 25ml volumetric flask, dissolved in 10ml of solvent and made up to the mark with solvent to obtain final concentration of 1000µg/ml ($1^o$ stock solution). From the $1^0$ stock solution, 1.0ml was pipetted out and transferred into a 10ml volumetric flask and made up to the mark with solvent to obtain a final concentration of 100µg/ml (standard $2^0$ stock solution).

***Selection of Analytical Wave Length:*** From the standard $2^0$ stock solution, 10 µg/ml working standard solution was prepared by 1.0ml was pipetted out and transferred in to a 10ml volumetric flask and made up to the mark with solvent and scanned in the wave length range of 200-400nm and maximum absorbance was found at 254nm.

***Selection of Analytical Concentration Range:*** Appropriate aliquots were pipetted out from the $2^0$ stock solution in to a series of 10 ml volumetric flasks. The volume was made up to the mark with

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solvent to get a set of solutions having the concentration range, ranging from 3, 6, 9, 12, 15, 18µg/ml. Absorbances of the above solutions were measured at 254nm. The concentrations showing absorbance within 1 was selected as analytical concentration range.

***Molar Absorptivity:*** The absorbance of all the concentrations was determined in the entire linearity range. The molar absorptivity was determined using the formula;

A = abc

Where,

A = Absorbance of solution

a = absorptivity (ε, $Lmol^{-1}cm^{-1}$)

b= path length (sometimes written as't' (thickness of the cell in cm)

c = concentration of solution (g/100ml)

***Sandell's Sensitivity:*** Sandell's sensitivity is calculated by using the formula

S= εs .y

Where,

S= sandell's sensitivity

εs= specific extinction coefficient

y= concentration of the substance in mg/lt

***Analysis of Tablet Formulation:*** Marketed tablet formulation (ENTAVIR) containing 1.0mg of entecavir was analyzed by this method. Twenty tablets were accurately weighed and average weight was determined. The tablets were crushed in to fine powder and a powder equivalent to 10 mg of pure drug was weighed and transferred in to 100ml volumetric flask, dissolved in 50ml of solvent and sonicated for 2 min. The solution was filtered through whatmann filter paper. The residue was washed with 10ml portions of solvent three times and the total volume of the filtrate was made up to the mark solvent to obtain 100µg/ml ($1^o$ stock solution). From the $1^o$ stock solution, 1.0ml was pippeted out and transferred into a 10ml volumetric flask and the volume was made up to the mark with solvent to obtain the final concentration of 10µg/ml. Three replicates of above concentration were prepared and the absorbances were analyzed at 254nm. Then the concentration of the above solution was determined by substituting the absorbance value in regression equation method and the percentage purity was determined.

***Method Validation:***

The developed method was validated according to ICH (Q2B) guidelines. The following validation parameters linearity, range, accuracy, precision, limit of detection, limit of quantitation and ruggedness were carried out.

***Linearity and Range:*** To establish the linearity of proposed method, aliquots of $2^o$ stock solution 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8ml were pippeted out and transferred into a series of 10ml volumetric flasks and the volume was made up to the mark with solvent to obtain final concentration of 3,6,9,12,15 and 18µg/ml. Absorbances of the above solutions were measured at 254 nm. The drug follows the Beer-Lambert's law within the concentration range of 3-18µg/ml.A calibration curve of concentration Vs. absorbance was plotted. The regression equation was established and correlation coefficient was determined.

***Accuracy:*** The interference of excipients in tablet additives was tested for the application of the proposed method to commercial formulation. To confirm the accuracy of the proposed method, recovery experiments were performed by standard addition technique. In this method a known quantity of pure drug was added to the pre-analyzed sample solutions at three different levels i.e. 80 %, 100% and 120% of the label claim.

***Procedure***

***Preparation of 80% Recovery Sample:*** 0.8ml of $2^o$standard stock solution (8µg/ml) was added to the 0.5ml (5µg/ml) of $1^0$sample stock solution in a 10ml volumetric flask and diluted up to the mark with solvent to get 80% recovery sample (13µg/ml).

***Preparation of 100% Recovery Sample:*** 1.0ml of $2^0$standard stock solution (10µg/ml) was added to the 0.5ml (5µg/ml) of $1^0$sample stock solution in a 10ml volumetric flask and diluted up to the mark with solvent to get 100% recovery sample (15µg/ml).

***Preparation of 120% Recovery Sample:*** 1.2ml of $2^0$standard stock solution (12µg/ml) was added to the 0.5ml (5µg/ml) of $1^0$sample stock solution in a 10ml volumetric flask and diluted up to the mark with solvent to get 120% recovery sample (17µg/ml).

At each level of recovery studies, three determinations were performed. The results obtained were compared with expected results and the values of percent recovery were calculated.

***Precision:*** The precision of an analytical method is the degree of agreement among the individual test results obtained when the method is applied repeatability to multiple sampling of the same homogenous sample under prescribed conditions.

***Procedure***

***Repeatability:*** In intra-day precision a set of six determinations containing 18µg/ml (100% concentration) were prepared and analyzed at 254nm on the same day and %relative standard deviation (%RSD) was calculated.

***Intermediate Precision:*** In inter-day precision a set of six determinations containing 18µg/ml were prepared and analyzed at same time on three

different days at their selected analytical wavelength of 254nm. The variation of the results on different days was analyzed and %RSD was calculated.

***Limit of Detection (LOD):*** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The Limit of detection was determined by using calibration standards.
The limit of detection (LOD) may be expressed as;

$$LOD = 3.3 \text{ X} \frac{\sigma}{S}$$

***Limit of Quantitation (LOQ):*** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Limit of detection was determined by using calibration standards.

The limit of quantitation (LOQ) may be expressed as;

$$LOQ = 10 \text{ X} \frac{\sigma}{S}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve.

***Ruggedness:*** Working standard solution containing 15µg/ml was analyzed by a different analyst on same instrument on different days and the percent relative standard deviation (%RSD) was reported.

## RESULTS AND DISCUSSION

A simple, sensitive and economical UV-Spectrophotometric method was developed for the estimation of Entecavir in bulk and pharmaceutical dosage form. Accurate results were obtained by utilizing the proposed method for the quantitation of Entecavir and a good agreement with the results obtained by the reported method was found. The absorption spectra for entecavir were recorded in the wave length region of 200-400nm in UV method. In the proposed method, $\lambda_{max}$ of Entecavir was found to be 254nm. It was observed that the optimized method was linear within specific concentration and the absorption spectrum was reported in figure 2. The calibration curve was linear over the concentration range of 3-18µg/ml with correlation coefficient of 0.999 as shown in the figure 3 and the results were reported in table 1. Accuracy of the proposed method was examined by performing recovery studies by standard addition method for drug product. Percentage recovery of Entecavir ranges from 99.53-100.41%w/w indicating that the developed method is accurate and the % RSD was less than 2 which indicates a good accuracy of the method and the results were reported in table 2. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 6 determinations over 100% concentration which shows %RSD less than 2 indicates that the method was precise and the results were reported in table 3. The limit of detection and limit of quantitation were found to be 1.25µg/ml &3.81µg/ml. The results were reported in table 4. The proposed method was validated as per ICH ($Q_2A$) guidelines, and was applied for analysis of the same in marketed formulation. Recovery studies indicate the absence of interference from common pharmaceutical excipients. Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational conditions, the % R.S.D. reported was found to be less than 2 %. It does not show any statistical variation between the results. The results were reported in table 5. Marketed formulation was analyzed with the proposed method and the %purity was found to be 99.8%. The results were reported in table 6.

## CONCLUSION

The proposed, validated spectrophotometric method was highly simple, sensitive and economical. The developed method was validated as per the International Conference on Harmonisation ICH(Q2B) Guidelines, and were found to be applicable for routine quantitative analysis of Entecavir by in pharmaceutical dosage forms. This method has been found to be better than previously reported methods, due to its wider range of linearity, use of economical mobile phase, lack of extraction procedures. Hence, the above method can be used in quality control for routine analysis of finished products of Entecavir without any interference.

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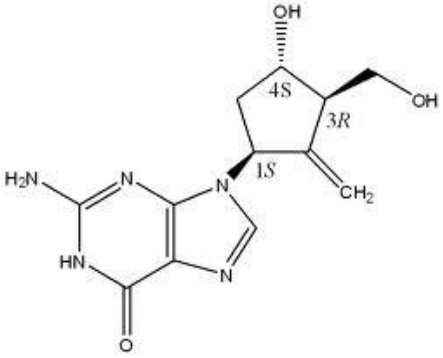


**Fig1: Structure of Entecavir**

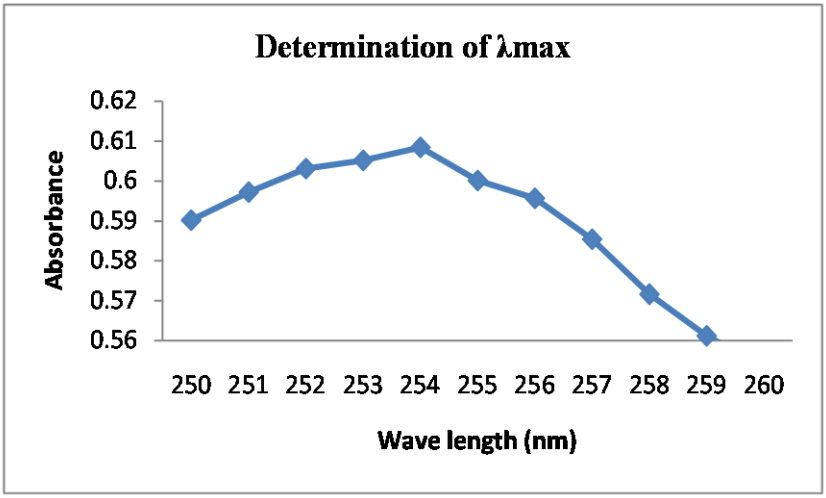


**Figure2: Absorption Curve of Entecavir**

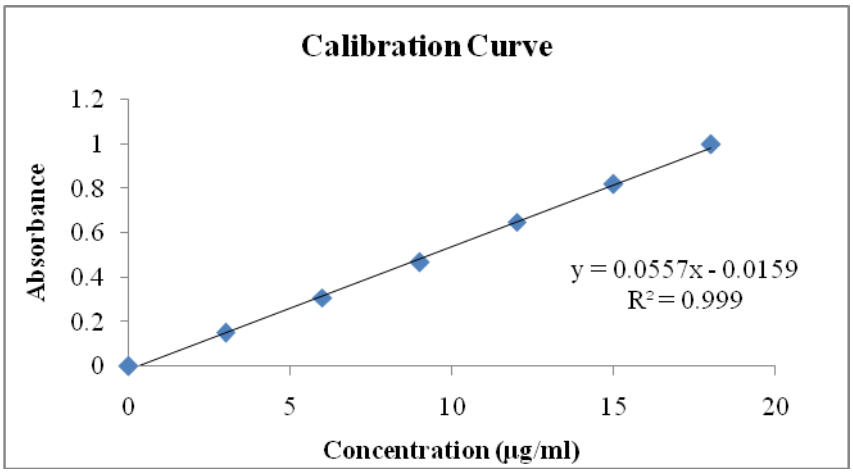


**Figure3: Calibration Curve of Entecavir**

**Table1: Linearity Data of Entecavir**

| Concentration (µg/ml) | Absorbance | | |
|---|---|---|---|
| | A1 | A2 | A3 |
| 3 | 0.1499 | 0.1621 | 0.1552 |
| 6 | 0.3089 | 0.3225 | 0.3126 |
| 9 | 0.4688 | 0.4851 | 0.4638 |
| 12 | 0.6495 | 0.6528 | 0.6124 |
| 15 | 0.8194 | 0.8132 | 0.7642 |
| 18 | 0.9987 | 0.9446 | 0.9218 |
| Slope | -0.0159 | 0.0531 | 0.0509 |
| Intercept | 0.0556 | 0.0043 | 0.0025 |
| Correlation coefficient (R) | 0.9990 | 0.9992 | 0.9999 |

**Table2: Percent Recovery Data of Entecavir**

| Recovery level (%) | Amount Added(µg/ml) | | Amount found(µg/ml) | Percent Recovery (%w/w) | %RSD |
|---|---|---|---|---|---|
| | Standard | Test | | | |
| 80 | 8 | 5 | 12.94 | 99.53 | 0.214 |
| 100 | 10 | 5 | 14.99 | 99.93 | 0.203 |
| 120 | 12 | 5 | 17.07 | 100.41 | 0.176 |
| Mean recovery (%w/w) | | 99.53-100.41 | | | |

%RSD: percent regression deviation

**Table3: Precision Data of Entecavir**

| Concentration (µg/ml) | Intra-day Precision | Inter-day Precision | | |
|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 |
| 25 | 0.9978 | 0.9972 | 0.9897 | 0.9956 |
| | 0.994 | 0.9964 | 0.9901 | 0.9886 |
| | 0.9948 | 0.9958 | 0.9942 | 0.9994 |
| | 0.9937 | 0.9946 | 0.9890 | 0.9987 |
| | 0.9973 | 0.9985 | 0.9920 | 0.9962 |
| | 0.9955 | 0.9924 | 0.9974 | 0.9872 |
| *SD | 0.0017 | 0.0021 | 0.0032 | 0.0052 |
| **Mean | 0.9955 | 0.9958 | 0.9921 | 0.9943 |
| %RSD | 0.171 | 0.2134 | 0.3247 | 0.5148 |

*standard deviation, **mean of six replications, %RSD: percent regression deviation

**Table4: LOD and LOQ Data of Entecavir**

| S.No | Parameters | Formula | Results |
|---|---|---|---|
| 1 | Limit of Detection (LOD) | 3.3xσ/S | 1.25 |
| 2 | Limit of Quantitation (LOQ) | 10xσ/S | 3.81 |

**Table5: Ruggedness (Analytical Variation) Data of Entecavir**

| S.No | Absorbance (15µg/ml) | | | |
|---|---|---|---|---|
| | Analyst 1 | Analyst 2 | Instrument 1 | Instrument 2 |
| 1 | 0.8194 | 0.8187 | 0.8096 | 0.8165 |
| 2 | 0.8092 | 0.8101 | 0.8164 | 0.8142 |
| 3 | 0.8021 | 0.7995 | 0.8214 | 0.8046 |
| 4 | 0.7985 | 0.8032 | 0.8054 | 0.8024 |
| 5 | 0.8125 | 0.8015 | 0.8018 | 0.8092 |
| 6 | 0.8012 | 0.7984 | 0.8142 | 0.8134 |
| *SD | 0.007 | 0.007 | 0.007 | 0.005 |
| **Mean | 0.807 | 0.805 | 0.811 | 0.810 |
| %RSD | 0.989 | 0.966 | 0.895 | 0.696 |

*standard deviation, **mean of six replications, %RSD: percent regression deviation

**Table6: Assay Data of Entecavir**

| Brand name | Absorbance | Label claim (mg) | Amount found | Percentage purity (%w/w) |
|---|---|---|---|---|
| Entavir | 0.5397 | 1.0 | 0.998 | 99.8 |

**Table7: Summarized Results of UV Spectrophotometric Method**

| **Validation Parameters** | **Results** |
|---|---|
| $\lambda_{max}$ (nm) | 254 |
| Linearity Range (µg/ml) | 2-18 |
| Correlation Coefficient (R) | 0.999 |
| Regression Equation | Y=0.0557x-0.0159 |
| Molar Absorptivity (Lt $mol^{-1}$ $cm^{-1}$) | 499.66 |
| Sandal's Sensitivity (µg$cm^{-2}$/0.001abs units) | 0.02013 |
| Accuracy (%w/w) | 99.53-100.41 |
| **Precision (%RSD)** | |
| Intra-Day | 0.171 |
| Limit of Detection (µg/ml) | 1.25 |
| Limit of Quantitation (µg/ml) | 3.81 |
| **Ruggedness (%RSD)** | |
| Analyst 1 | 0.989 |
| Analyst 2 | 0.966 |
| Instrument 1 | 0.895 |
| Instrument 2 | 0.696 |
| Assay (%w/w) | 99.82 |

**REFERENCES**


1. http://enwikipedia.org/wiki/entecavir.
2. Bertan G Katzaung. Basic and clinical pharmacology, 9th edition., 2004.
3. Sweetman SC. Martindale: The Complete Drug Reference, 35th edition, London Pharmaceutical press 2007: pp786.
4. Rajeswari M et al. Development of new visible spectrophotometric methods for the determination of Entecavir in pharmaceutical dosage forms. Int J Pharma & Bio Sciences 2011; 2(4):91-94.
5. KPR Chowdary, Devalarao G. Visible Spectrophotometric methods for the determination of Salmetarol xinafoate. Indian Journal of Pharmaceutical Sciences 1999; 61(4):246-47.
6. Devala Rao G et al. Visible Spectrophotometric Methods for the determination of Sparfloxacin in pharmaceutical formulations. The Eastern Pharmacist 1999:121.
7. KPR Chowdary et al. Determination of Lomefloxacin by using Ferric Alum Reagent. Journal of Institution of Chemists (India) 1999; 71(6):206-8.
8. Girish Kumar K et al. Spectrophotometric determination of Nimesulide using 3-methyl-2-benzothiazolinone hydrazone hydrochloride reagent. Indian Drugs 1999; 36(3):185.
9. Vijay Amirthraj R et al. Development and Validation of RP-HPLC For the Estimation of Entecavir in Tablet Dosage Form. International Journal of Reserch in Pharmaceutical and Biomedical Sciences 2011; 2(3):1033-40.
10. Jung BH et al. Simultaneous Determination of 17 Antiretroviral drugs in Human Plasma for Quantitative Analysis with Liquid Chromatography-Tandem Mass Spectrometry. Biomed Chromatography B 2007; 10:1095-1104.
11. Kalyana Chakravarthy V, Gowri Sankar D. LC Determination of Entecavir in Pharmaceutical Formulation. International journal of Pharma. Reserch & Development 2011; 2(3):233-41.
12. Satyanarayana L et al. The Estimation of Entecavir in Tablet Dosage Form by RP-HPLC. Research Journal of Pharmacy and Technology 2011; 4(11):1699-1701.
13. Dalmora SL et al. Validation of a stability indicating RP-HPLC method for the determination of Entecavir in tablet dosage form. J AOAC 2010; 93(2):523-30.
14. Elena Katz Roy Eksteen, Peter Schoenmakers. Hand Book of HPLC. Pearson Education Limited. Mumbai 1998; 78: pp143-7.
15. Malipatil SM et al. Validated UV-Spectrophotometric Estimation of Entecavir in Bulk and Formulations. RGHUS Journal of Pharmaceutical Science 2011; 1(2):111-6.
16. Vijay Amirthraj R et al. Development and Validation of UV-Spectrophotometric for the Estimation of Entecavir in Tablet Dosage Form. Journal of Pharmacy Reserch 2011; 4(3):1145-46.
17. Silversteen RM et al. Spectrophotometric Identification of Organic Compounds. Academic press, 4th Edn, New Delhi; 1981: pp 87-90.
18. Murali D et al. Spectrophotometric determination of etravirin in bulk and pharmaceutical formulations. AJAC 2014; 5(2):77-80.