Rp-Hplc Method Development for Estimation of Zidovudine in Tablets

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ABSTRACT: Analytical method is a specific application of a technique to solve an analytical problem. The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. The manufacture of materials, whose composition must be known precisely, is to be monitored by analytical instruments.

I.   INTRODUCTION:

Many of the reactions involve color or fluorescence of a particular drug are quite selective or can be rendered selective through the introduction of masking agents, control of PH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separation .

1.   This is preferably followed by general methodology for UV-Visible and HPLC method developments.

2.   Followed by literature of drugs used in Analysis.

HPLC is a modern technique, it is a much more reliable and reproducible method for the standardization of both single and compound formulation. HPLC is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption or ion exchange process, depending upon the size of stationary phase used.

HPLC is one of the most versatile instruments used in the field of pharmaceutical analysis. It provides the following features.

➢   High resolving power
➢   Speedy separation
➢   Continuous monitoring of the column effluent
➢   Accurate quantitative measurement
➢   Repetitive and reproducible analysis using the same column

➢   Automation of the analytical procedure and data handling.

DRUG PROFILE OF ZIDOVUDINE:
Zidovudine is an Nucleoside and Nucleotide Reverse Transcriptase used in the treatment of AIDS .
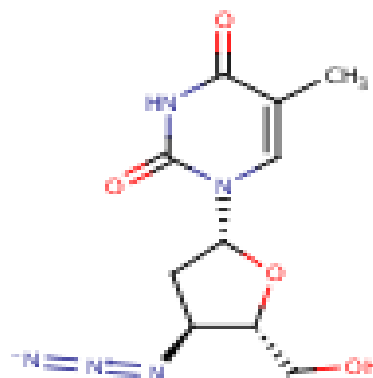
Systematic IUPAC name:
1-[(2R, 4S, 5S)-4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-1, 2, 3, 4-tetrahydropyrimidine-2,4-dione
Chemical data:
Formula   : $C_{10}H_{13}N_5O_4$
Mol. Mass : 267.2413 g/mol
Category   : Anti-HIV Agents
Physical state   : Solid, White Crystalline Powder
Solubility   : Freely soluble in water, methonal and acetonitrile
Melting point   : 106-112°C

Mechanism of action:
Zidovudine, a structural analog of thymidine, inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA.
Clinical use:
✓   Anti-HIV Agents
✓   Ant metabolites

✓ Nucleoside and Nucleotide Reverse Transcriptase Inhibitors
Adverse Effects:
Symptoms of overdose include fatigue, headache, nausea, and vomiting

METHOD DEVELOPMENT
Based on drug solubility and P^{ka} Value following conditions has been used to develop the method estimation of Zidovudine**.**
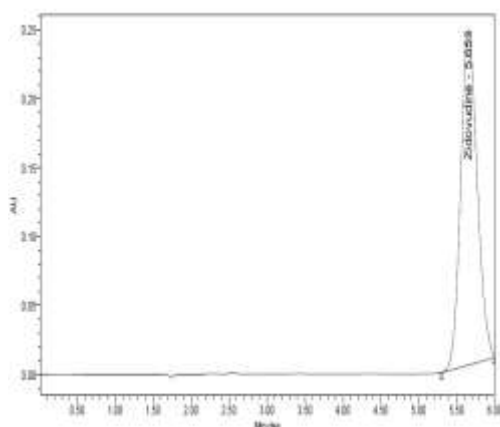
Trial 1

Fig No 1 Chromatogram of Trial 1

Chromatographic Conditions:
Column: Kromocil-(150*4.6 μm)
Mobile phase : Buffer: Methanol (80:20)
Buffer : 0.02M Sodium acetate P^H 5.5 adjusted with Glacial acetic acid
Flow rate : 1.0 ml/min
Detector: UV at 266nm
Run time: 20 minutes
Diluent : Water: Methanol (90:10)
Temperature : 25^0C
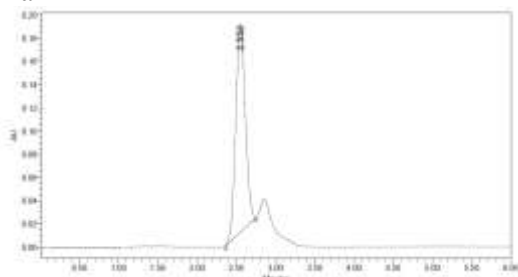**Injection Volume** : 20μL

Trial 2

Fig No 2 Chromatogram of Trial 2

Column : Kromocil-(150*4.6 μm)
Mobile phase : Buffer: Methanol (70:30)

Buffer : 0.02M Sodium acetate P^H 5.3 adjusted with Glacial acetic acid
Flow rate : 1.3ml/min
Detector : UV at 266nm
Run time: 20 minutes
Diluent : Methanol: Water (10:90)
Temperature : 25^0
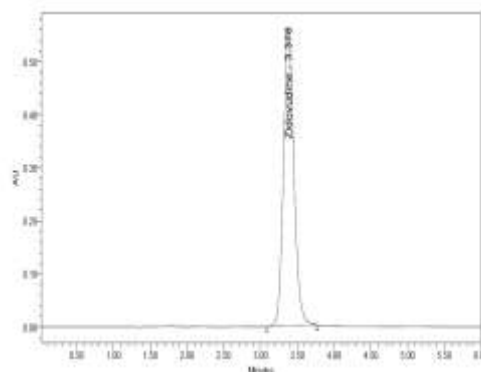**Injection Volume** : 20μL
Trial 3

Fig No 3 Chromatogram of Trial 3

Column : Kromocil-(150*4.6 μm)
Mobile phase : Buffer: Methanol (60:40)
Buffer : 0.02M Sodium acetate P^H 5.3 adjusted with Glacial acetic acid
Flow rate : 1.3ml/min
Detector : UV at 266nm
Run time: 20 minutes
Diluent: Methanol: Water (10:90)
Temperature : 25^0
Injection Volume : 20μL

Preparation of Mobile phase:
Mobile Phase: Buffer: Methanol (60:40)

Buffer Preparation:
Dissolve 3gm of sodium acetate 900 mL of water and mix. Adjust the pH of this solution to 5.3 (±0.05) with glacial acetic acid.
Diluent: Methanol: Water (30:70)

Stock and Standard Solution Preparation:
Weigh accurately about 50mg Zidovudine working standard and transfer into a 50 mL volumetric flask, add 35 mL of diluent and sonicate to dissolve for about 5 min, further made up the volume with diluent (Stock Solution). From stock solution 2 ml was taken in 10 ml volumetric flask and volume was made up to the mark with diluent (Standard Solution).

Preparation of Linearity Solutions
Preparation of 50% Solution (100ppm):
From stock solution 1 ml was taken into the 10 ml volumetric flask and volume was made up with diluent
Preparation of 75% Solution (150ppm):
From stock solution 1.5 ml was taken into the 10 ml volumetric flask and volume was made up with diluent
Preparation of 100% Solution (200ppm):
From stock solution 2 ml was taken into the 10 ml volumetric flask and volume was made up with diluent
Preparation of 125% Solution (250ppm):
From stock solution 2.5 ml was taken into the 10 ml volumetric flask and volume was made up with diluent
Preparation of 250% Solution (300ppm):
From stock solution 3 ml was taken into the 10 ml volumetric flask and volume was made up with diluent

Sample Preparation:
Weigh 20 tablets and crush into powder. Weigh powder equivalent to 500 mg of the Zidovudine and transfer into a 100 mL volumetric flask, add 70 mL of diluents and sonicate for 15 min , further make up the volume with diluent. Further dilute the filtrate 4 mL to 100 mL with diluent.

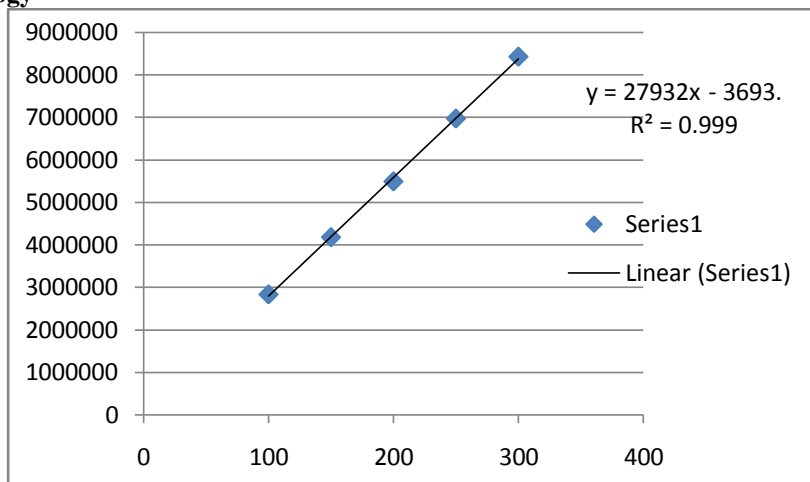Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae.
Calculate the percentage purity of Zidovudine present in tablet using the formula:
Calculation:

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std. Dil. Fac}}{\text{Spl. Dil. Fac}} \times \frac{\text{Avg. Wt of Tab}}{\text{L.C}} \times \text{Potency of Std}$$

Spl area – Sample Peak area
Std area – Standard Peak area
Std. Dil. Fac
Spl. Dil. Fac
Avg. Wt of Tab
L.C
Potency of Std

II. RESULTS AND DISCUSSIONS:
Zidovudine is the drug mainly used to treat AIDS. A simple reverse phase HPLC method was developed for the determination of Zidovudine. Column Kromocil (150 x 4.6 mm, packed with 5μm) with mobile phase Buffer: Methanol (60:40) was used. The flow rate was 1.3ml/ min and effluent was monitored at 266 nm. The column temperature was 25°C. The Retention time was found to be3.396..

Assay Methodology

y = 27932x - 3693.
R² = 0.999

Fig No:04 Graph Showing Linearity

Assay of Zidovudine : From the Chromatograms it was found that the sample and standard retention times are similar i.e3.378 to 3.788. From linearity Table 1, it was found that the drug obeys linearity within the concentration range of 100-300ppm for zidovudine By using this method assay of marketed formulated was carried out.

III. SUMMARY AND CONCLUSION

Zidovudine is the drug used in the treatment of AIDS. It is a nucleoside & nucleotide reverse transcriptase inhibitor.

From literature review and solubility analysis initial chromatographic conditions Mobile phase acetate buffer: methanol 20:80 were set (Buffer P^H 5.3 adjusted with glacial acetic acid), Kromocil 150*4.6mm Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 266 nm. As the methanol content was increased Zidovudine got eluted with good peak symmetric properties. Mobile phase buffer: Methanol (60:40), Column Kromocil 150*4.6mm 5μm and flow rate 1.3ml/min, detection wave length 266nm, column temperature 25^oC and diluent water: methanol (90:10) conditions were finalized as optimized method.

System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria.

Full length method was not performed; if it is done this method can be used for routine analysis of Zidovudine

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