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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF ZIDOVUDINE IN BULK AND IN CAPSULE FORMULATION

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ABSTRACT: Zidovudine is Anti-HIV agent, Antimetabolites and Nucleoside and Nucleotide Reverse Transcriptase Inhibitors medication used in the treatment of HIV infection. A simple, selective, precise, accurate and cost effective RP HPLC method has been developed and validated for estimation of Zidovudine Bulk and in dosage form. In the chromatographic conditions, stationary phase is phenomenex® C18 (250 X 4.6 mm, 5 µm) stationary phase with mobile phase consisting of mixture of water and methanol in the ration of (60: 40 v/v) was used at a flow rate of 1.0 mL/min. and column temperature was maintained ambient. Zidovudine detected at 266 nm by using PDA detector. Injection volume is 20µl. The chromatographic procedure separated Zidovudine and potential interfering peaks in an analysis time of 6 min. with Zidovudine eluting at about 4.5 min. The Peak purity plot of Zidovudine with purity index 0.99974. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Zidovudine in bulk and in capsule formulation. **Keywords:** Zidovudine, Validation, RP-HPLC.

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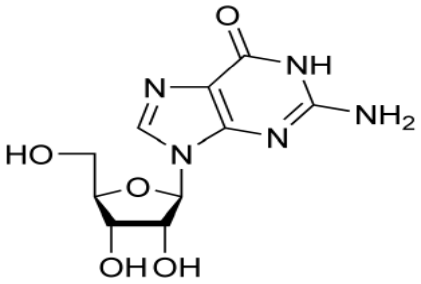
1. INTRODUCTION

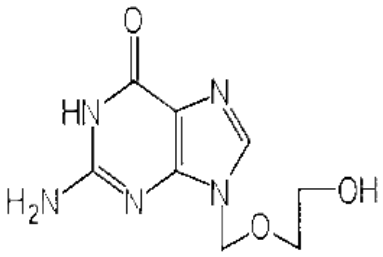
Zidovudine A dideoxynucleoside compound in which the 3'-hydroxy group on the sugar moiety has been replaced by an azido group [1]. This modification prevents the formation of phosphodiester

linkages which are needed for the completion of nucleic acid chains. The compound is a potent inhibitor of HIV replication, acting as a chain- terminator of viral DNA during reverse transcription. It improves immunologic function, partially reverses the HIV-induced neurological dysfunction, and improves certain other clinical abnormalities associated with AIDS. Its principal toxic effect is dose-dependent suppression of bone marrow, resulting in anemia and leukopenia. *It is used to treat HIV disease. The chemical name of Zidovudine is* 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione. Zidovudine is a NRTI with activity against HIV-1. Zidovudine is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV RT enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Zidovudine, a structural analog of thymidine, inhibits the activity of HIV-1 RT both by competing with the natural substrate dGTP and by its incorporation into viral DNA.

ANTIVIRAL DRUGS



Guanosine



The Guanosine analogue Acyclovir

Antiviral drugs are often nucleoside analogues, (fake DNA building blocks), which viruses mistakenly incorporate into their genomes during replication. The life-cycle of the virus is then halted because the newly synthesized DNA is inactive. This is because these analogues lack the hydroxyl groups, which, along with phosphorus atoms, link together to form the strong "backbone" of the DNA molecule. This is called DNA chain termination. Examples of nucleoside analogues are acyclovir for Herpes simplex virus infections and Lamivudine for HIV and Hepatitis B virus infections. Acyclovir is one of the oldest and most frequently prescribed antiviral drugs. Other antiviral drugs in use target different stages of the viral life cycle. HIV is dependent on a proteolytic enzyme called the HIV-1 protease for it to become fully infectious. There is a large class of drugs called protease inhibitors that inactivate this enzyme. A deep literature study reveals that the drug Didanosine is a NRTI**.** It inhibits viral RNA- dependent DNA polymerase (reverse transcriptase) and is incorporated into viral DNA (they are chain terminating

drugs). Stavudine and Zidovudine also belong to the same category with similar mechanism of action. Antiretroviral combination therapy defends against resistance by suppressing HIV replication as much as possible. Combinations of antiretrovirals create multiple obstacles to HIV replication to keep the number of offspring low and reduce the possibility of a superior mutation. If a mutation that conveys resistance to one of the drugs being taken arises, the other drugs continue to suppress reproduction of that mutation. With rare exceptions, no individual antiretroviral drug has been demonstrated to suppress an HIV infection for long; these agents must be taken in combinations in order to have a lasting effect. As a result, the standard of care is to use combinations of antiretroviral drugs. The results of Phase 2 Clinical Trial evaluate the dose ranging, safety and efficacy for Atazanavir at three doses in combination with Didanosine and Stavudine in Antiretroviral-Naive Subjects. [6] Another study demonstrates the phenotypic and genotypic resistance patterns of HIV-1 isolates derived from individuals treated with Didanosine and Stavudine. [7] The virological and immunological efficacy of the triple regimen containing Nevirapine (once or twice daily), Didanosine (once daily) and Stavudine, in antiretroviral-naive patients infected with HIV-1, was evaluated in an open-label, prospective, non-randomized, multi-centre, 52-week study. Combination therapy with the three reverse transcriptase (RT) inhibitors Stavudine, once- daily Didanosine and either once- or twice-daily nevirapine could be considered as an alternative option for first-line antiretroviral therapy. [8] Combination therapy with Stavudine plus Didanosine is safe and well-tolerated in HIV-infected children, producing durable, but incomplete, suppression of virus replication. This combination of nucleoside antiretroviral agents may provide a valuable backbone to protease inhibitor- containing treatment regimens for HIV-infected children. [9] A combination of Zidovudine, Didanosine, and Lamivudine was used to treat 10 patients with primary human immunodeficiency virus type 1 (HIV-1) infection 5-28 days after the onset of symptoms and the study concludes that triple-drug therapy has a potent antiviral effect during primary HIV-1 infection. [10] Combination therapy with Zidovudine and Didanosine selects for Zidovudine-resistant HIV-1 strains lacking a Didanosine resistance mutation and for multi drug-resistant strains containing novel pol mutations. [11] The combination of Zidovudine and Didanosine was well-tolerated at doses as high as those used in single agent therapy. Potent in vivo antiviral activity was observed. Combination therapy with nucleoside analogues may be an important approach to optimizing the use of these agents in the treatment of HIV infection. [12] There are a lot of studies going on for the use of Didanosine along with other NRTIs like Stavudine, Lamivudine and Zidovudine for effective combination therapy for the treatment of HIV infected patients. Hence many possibilities are there that fixed dose combinations for above mentioned drugs may be available in near future. Hence efforts were made to develop simple RP-HPLC methods for the simultaneous determination of Didanosine with Stavudine and Didanosine with Zidovudine. These methods can also be used in

various research studies and clinical trials where there is a need to analyze these drugs simultaneously. Several methods have been reported for the analysis of Zidovudine using High Performance Liquid Chromatography (HPLC) [14, 17, 18, 19]. Human Plasma and Urine by HPLC [13,20]. Tendem mass spectrometric for the simultaneous and Human plasma by HPLC [15]. Reported methods involved complicated time-consuming multi-step liquid-liquid extraction techniques [16]. To the best of our knowledge, there is no work in the literature reported about the estimation of Zidovudine from pharmaceutical formulation by using RP-HPLC. The purpose of this investigation was the development of a rapid, sensitive and validated HPLC method for quantification of Zidovudine from capsule forms.

THERAPY OF HIV INFECTION

- Nucleoside-Analogue Reverse Transcriptase Inhibitors (NRTI).
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs).
- Protease Inhibitors

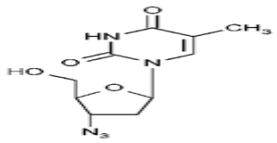
High Performance Liquid Chromatography (HPLC) [2]

A 'regulatory analytical procedure' is used to evaluate a defined characteristic of the drug substance or drug product. An 'alternative analytical procedure' is proposed by the applicant for use other than regulatory analytical procedure. [2] The modern methods of choice for quantitative analysis are HPLC, GC, and HPTLC, which are highly sophisticated. Chromatographic methods are commonly used in regulatory laboratories for the qualitative and quantitative analysis of drug substances, drug products, raw materials and biological samples throughout all phases of drug development, from research to quality control. HPLC is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. The rapid growth of HPLC has been facilitated by the development of reliable, moderately priced instrumentation and efficient columns. HPLC is an advanced form of liquid chromatography that is used to separate complex mixtures of molecules in chemical and biological systems. Compared to other chromatography systems, HPLC systems have higher resolution, faster cycle times and columns that can be reused without repacking or regeneration. The mobile phase of these systems can also be varied during the analysis, resulting in a gradient elution. HPLC columns have a stationary phase consisting of very small particle sizes with large surface areas that allow the application of high pressure to the solvent flow. The advantage of a well-accepted analytical technique like HPLC is that researchers develop variations of these systems that work particularly well for their specific applications. Normal phase was the first technique developed, which uses a polar stationary phase and a non-polar mobile phase, allowing it to work well for relatively polar analytes. Reversed phase HPLC, however, is the most commonly

used version. These systems have a non- polar stationary phase (i.e., treated silica) and an aqueous, moderately polar mobile phase. In reversed phase, retention times can be longer for molecules that are more non- polar, while polar molecules elute more readily. HPLC systems are widely used because of their accurate and reproducible results, high resolution, high precision, high sensitivity and ease of being automated to further reduce the attendant costs and time requirements.

2. MATERIALS AND METHODS

Table No.1: DRUG PROFILE OF ZIDOVUDINE *[1]*

DRUG PROFILE OF ZIDOVUDINE	
Description	A dideoxynucleoside compound in which the 3'-hydroxy group onthe sugar moiety has been replaced by an azido group. It is used to treat HIV disease.
IUPAC name	1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione
Chemical Formula	$C_{10}H_{13}N_5O_4$
CAS Reg. No.	30516-87-1
Solubility in water	10-50 g/L at 17 °C
Chemical structure	
Mol. Weight	267.2413 g/mol
Melting point	106-112 °C
Drug category	Anti-HIV Agent Antimetabolites Nucleoside and Nucleotide Reverse Transcriptase Inhibitors
Indication	For Treatment of HIV infections.

- **DRUG USED:**

Table No.2: DRUGS USED

DRUGS USED		
SR. NO.	**DRUGS**	**MANUFACTURER NAME**
1.	**ZIDOVUDINE**	EMCURE PHARMA, PUNE

➢ **REAGENT USED:**

Table No.3: REAGENT USED

LIST OF REGENTS USED			
SR. NO.	**CHEMICALS**	**MANUFACTURER NAME**	**GRADE**
1.	**METHANOL**	S.D. FINE CHEMICAL	HPLC
2.	**ACETONITRILE**	S.D. FINE CHEMICAL	HPLC
3.	**WATER**	FINAR	HPLC
4.	**AMMONIUM FORMATE**	S.D. FINE CHEMICAL	HPLC
5.	**FORMIC ACID**	S.D. FINE CHEMICAL	HPLC
6.	**GLACIAL ACETIC ACID**	S.D. FINE CHEMICAL	HPLC

➢ **EQUIPMENT AND APPARTUS USED:**

Table No.4: EQUIPMENT AND APPARATUS USED

EQUIPMENT AND APPARATUS USED			
SR. NO.	**INSTRUMENT NAME**	**MODEL NUM. & SOFTWARE**	**MANUFACTURER NAME**
1.	**HPLC**	LC-10AT, LAB SOLUTION	SHIMADZU, JAPAN
2.	**UV-VISIBLE SPECTROPHOTOMETER**	PHARMASPEC-1700, UV PROBE 2.0	SHIMADZU, JAPAN
3.	**ULTRASONIC BATH**	FRONTLINE FS4 ULTRASONIC CLEANER	MUMBAI
4.	**pH meter**	SYSTRONIC	MUMBAI
5.	**ELECTRONIC BALANCE**	CP2245S ANALYTICAL BALANCE	GOTTINGEN, GERMANY
6.	**0.45 µ HPLC FILTER**	-	MERCK
7.	**WHATMANN FILTER PAPER NO. 41**	-	MERCK
8.	**VOLUMETRIC FLASK**	-	BOROSIL

9.	**GRADUATED PIPETTE**	-	BOROSIL
10.	**MEASURING CYLINDER**	-	BOROSIL
11.	**FUNNEL**	-	BOROSIL
12.	**CONICAL FLASK**	-	BOROSIL
13.	**GLASS BEAKER**	-	BOROSIL
14.	**PLASTIC BEAKER**	-	BOROSIL

EXPERIMENTAL

➢ PREPARATION OF SOLUTIONS

Preparation of the mobile phase

HPLC grade solvents were used in separate bottle of gradient pumps as mobile phase. A mixture of water and methanol in the ratio of 60:40 v/v were adjusted by gradient pump operated by LC solution software. Mixed solvents were filtered through nylon 0.45 µm membrane filter and degassed by the instrument and used as mobile phase.

Preparation of standard solution (100 µg/mL)

Accurately weighed 10 mg Zidovudine was transferred to 100 mL volumetric flask, dissolved in 50 mL methanol and diluted up to mark with methanol to prepare standard solution having concentration of 100µg/mL

Preparation of sample solution

Twenty capsules were taken and accurate quantity of capsule content equivalent to 10 mg of Zidovudine was weighed and transferred to 100 mL volumetric flask, dissolved in methanol (60 mL) and sonicated for 30 min. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The solution was diluted up to the mark with methanol.

Determination of wavelength of maximum absorbance

The standard solution of Zidovudine (4.0 µg/mL) was scanned in the range of 200 to 400 nm and the UV spectrum was recorded.

➢ CHROMATOGRAPHIC CONDITIONS

The chromatographic separations were performed using the final chromatographic conditions as mentioned in table 5.

Table No.5: Final chromatographic conditions

SR. NO.	PARAMETER	DESCRIPTION
1.	Stationary Phase	Phenomenex® C_{18} column with 250 mm x 4.6 mm i.d. and 5 µm particle size
2.	Mobile Phase	Mixture of water and methanol in the ratio of 60:40 v/v

3.	Flow Rate	1.0 mL/min
4.	Detection wavelength	266 nm
5.	Detector	PDA detector
6.	Injector	Manual injector loop
7.	Injection volume	20 µL
8.	Column Temperature	Ambient
9.	Run Time	06 min
10.	Diluent	Methanol

METHOD VALIDATION [2]

- **Linearity and range**

Accurately measured standard solutions of Zidovudine (0.001, 0.05, 0.2, 0.4, 0.6, 0.8 and 1.0 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with methanol. 20 µL each of these solutions were injected using manual injector loop under the final chromatographic conditions described above. Calibration curve was constructed by plotting peak area versus concentration of Zidovudine and the regression equation was calculated.

- **Accuracy (% Recovery)**

The accuracy of the method was determined by calculating recoveries of Zidovudine by the standard addition method. For this known amount of standard solutions of Zidovudine (75, 100, and 125 % level) were added to pre analyzed sample solutions. The amount of Zidovudine was analyzed by using the regression equation of the calibration curve.

- **Precision**

Method precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) standard solution of Zidovudine (4 µg/mL). The results were reported in terms of % CV.

Intermediate precision

Intermediate precision was evaluated in terms of intraday and inter day precision. The intraday precision was investigated by analyzing three different standard solutions of Zidovudine (3.0, 7.0 and 9.0 µg/mL). The inter day precision was investigated by analyzing three different standard solutions of Zidovudine (3.0, 7.0 and 9.0 µg/mL) on different days. The results were reported in terms of %CV.

- **Robustness**

Method robustness was performed by applying small changes in the composition of mobile phase, analytical wavelength and flow rate. Robustness of the method was done at three different concentration levels of 3.0, 7.0 and 9.0 µg/mL for Zidovudine. The results were expressed in terms of % Recovery ± S.D.

- **LOD and LOQ**

The LOD and LOQ of the method were determined by using the following equations:

$$LOD = 3.3\ \sigma / S \text{ AND } LOQ = 10\ \sigma / S$$

Where, σ = standard deviation of the response and

S = slope of the calibration curve

- **Specificity and selectivity**

The specificity of the method was established through resolution factor of the drug peak from the nearest resolving peak and also among all other peaks. Selectivity was confirmed through peak purity data using a PDA detector. To assess the method specificity, powder without Zidovudine (placebo) was prepared with the excipients as required for commercial preparation and compared with respective drug standard to evaluate specificity of the method. Representative chromatograms of placebo and standard were compared for retention time, resolution factor and purity.

- **System suitability**

The system suitability parameters like theoretical plates (T_p), and asymmetry factor (As), capacity factor (K'), resolution (R_S), retention time (RT) and tailing factor (T_f) were calculated by Class VP LC solution software. The HPLC system was equilibrated with the initial mobile phase composition, followed by six injections of the standard solution having same concentration. These six consecutive injections were used to evaluate the system suitability on each day of method validation. In order to establish system suitability for the instrument, six consecutive injections of Zidovudine were prepared from the standard solution and analyzed.

- **Solution stability**

The solution stability of Zidovudine in the proposed method was carried out by leaving both the sample and standard solution in tightly capped volumetric flask at room temperature for 24 hours. The same sample solutions were assayed for interval of 6 hours up to the 24 hours. Both the solution was prepared in methanol as solvent and mixture of water and methanol in a ratio of 60:40 v/v as mobile phase. The obtained results were compared with the freshly prepared solution.

- **Analysis of Zidovudine in Capsule formulation**

Appropriate three different aliquots from sample solution were suitably diluted with methanol in such a way to get concentrations in a range of 0.01 to10.0 µg/mL for Zidovudine. The finally prepared solutions were analyzed under chromatographic condition as described in table no. 5. The amount of Zidovudine present in sample solution was determined by substituting the area response into the regression equation of both the drugs in the method.

➢ **Comparison of the developed method with the official method** [3,4,5]

The drug is official in I.P. The developed method was compared with the official method and the results were analyzed using t- test.

3. RESULTS AND DISCUSSION

➢ **Determination of Wavelength maxima**

Solution of Zidovudine was scanned between 200 and 400 nm with the help of UV spectrophotometer. From the UV spectra it was observed that the maximum response is obtained at 266 nm. Hence 266 nm was selected as the analytical wavelength. The UV spectrum is shownin Figure 1.

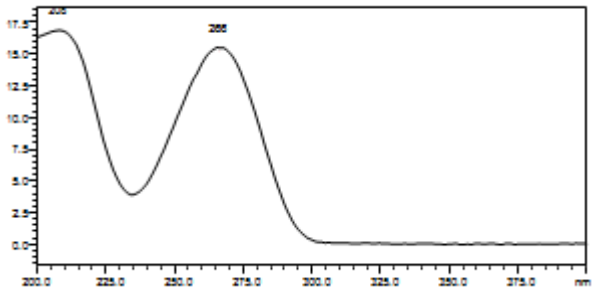


Figure 1: UV spectrum of Zidovudine

➢ **Method optimization**

The aim of this study was to develop a gradient RP-HPLC assay method for the analysis of Zidovudine in bulk and in capsule formulation. Since the drug is soluble in polar solvents like methanol and water, a RP-HPLC method was thought to be suitable. A Phenomenex C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was preferred over other columns because it has high carbon loading with very closely packed material to give high performance over other C_{18} columns for reverse phase HPLC analyses. Initial studies to optimize the mobile phase are shown in table No.6.

Table No.6: Results of the initial trials for optimization of mobile phase

Mobile phase	Ratio	Result
A mixture of methanol and water	10:90	No retention of drug
A mixture of ACN and methanol	50:50	No peak observed
A mixture of water and methanol	50:50	Broad peak observed
A mixture of water and methanol	60:40	Sharp peak and good resolution

Finally, acceptable resolution with reasonable peak shapes and high peak purity was achieved by using a mixture of water and methanol in the ratio of 60:40 v/v with flow rate of 1 mL/min at 266 nm. The method parameters were optimized for the analysis of Zidovudine in capsule formulation. A representative chromatogram is shown in Figure 2, which satisfies all the system suitability criteria, better resolution of the peak from solvent peak with clear base line separation.

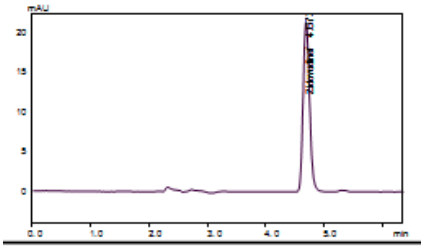


Figure 2: Representative chromatogram showing peak of Zidovudine (8µg/mL) at 266nm

METHOD VALIDATION [2]

The developed method as described above was validated for various parameters like system suitability, specificity, linearity, precision, accuracy, LOQ and LOD.

- **Linearity and range**

Linearity of the method was evaluated at seven concentration levels by diluting the standard solution in the concentration range of 0.01 - 10 µg/mL for Zidovudine. The results show that an excellent correlation existed between the peak area and concentration of analyte. The calibration curve was prepared by plotting the peak area versus the concentration and the regression equation was calculated. The calibration curve was repeated for five times and the average results are mentioned in table 7. Figure 3 shows the calibration curve of Zidovudine for LOD and LOQ and table 8 gives optical regression characteristics for analysis of Zidovudine by RP-HPLC method.

Table 7: Data for calibration curve for Zidovudine

Conc. of Zidovudine µg/mL	Peak area	%CV
0.01	3004	1.572
0.5	20475	1.043
2	50476	1.201
4	81837	0.760
6	112635	1.001
8	149354	0.658
10	180827	1.254

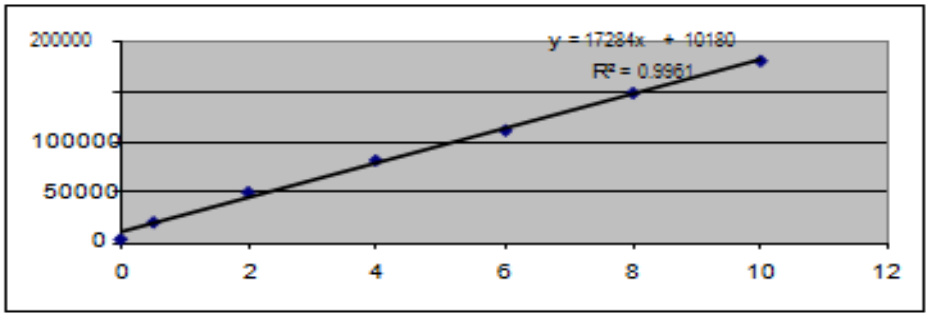


Figure 3: Calibration curve of Zidovudine

Table 8: Optical and regression characteristics for analysis of Zidovudine

Parameters	Zidovudine
Concentration range (µg/mL)	0.01 – 10
LOD (µg/ml)	0.0034
LOQ (µg/ml)	0.0102
Regression Equation	Y= 17284x 10180
Correlation coefficient (R^2)	0.9961

➢ **Precision**

Method precision

The results of repeatability experiment are shown in table 9. Method precision was determined by repeatedly injecting 4.0 µg/mL concentration of Zidovudine (n = 6). The developed method was found to be precise and the results are reported in terms of % CV.

Table 9: Method precision data of Zidovudine by RP-HPLC method

Drug	Concentration	RT	%CV	Area	%CV
Zidovudine	4.0µg/mL	4.657	0.654	82012	0.756

Intermediate precision

The results of intermediate precision experiment for both intraday and inter day are shown in table 10. Replicate analyses of three concentrations of the standard solution show good reproducibility. The results are reported in terms of % CV values.

Table 10: Intermediate precision data of Zidovudine by RP-HPLC method

Zidovudine µg/mL	Intra-day measured mean area, % CV (n=6)	Zidovudine µg/mL	Inter-day measured mean area, % CV (n=6)
3	61623, 1.271	3	61487, 1.675
7	130758, 0.586	7	130471, 1.004
9	164573, 1.100	9	165453, 1.501

➢ **Accuracy (% Recovery)**

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of drug (70, 100, and 125 %) was added to the pre analyzed sample solution. This solution was analyzed under the chromatographic conditions mention in table no.5. The assay was repeated over 3 consecutive days to obtain intermediate precision data. Results of accuracy are shown in table 11.

Table 11: Accuracy (% Recovery) study of Zidovudine by RP-HPLC method

Drug	Known	Added	% Recovery ± S.D.
Zidovudine	4	3 (75%)	101.3544 ± 1.0352
	4	4 (100%)	99.0342 ± 1.1253
	4	5 (125%)	98.2543 ± 1.0004

➢ **Robustness**

To evaluate the robustness of the proposed method, experimental conditions were deliberately altered and the response of the drugs was recorded. The results of minor variations in composition of mobile phase, wavelength, and flow rate are shown in table 12.

Table 12: Robustness data of Zidovudine (6 µg/mL) by RP-HPLC method

Parameter	Modification	% Recovery ± S.D. (n=6)
Flow rate (1 mL/min)	+ 0.1	99.2638 ± 0.8238
	- 0.1	99.1461 ± 1.1231
Mobile phase composition	59:41	99.1632 ± 0.9812
	61:39	98.9651 ± 1.1138
Wave length (266 nm)	+ 1	99.4632 ± 0.6728
	- 1	99.7968 ± 0.8946

➢ **LOD and LOQ**

These data show that the method is sensitive for the determination of Zidovudine. The LOD and LOQ were calculated by using the equations mentioned in table no.5. The results are shown in table 13.

Table 13: LOD and LOQ for Zidovudine

	σ	Slope	LOD µg/mL	LOQ µg/mL
Zidovudine (7 µg/mL)	17.5635	17204	0.0034	0.0102

➢ **Specificity and selectivity**

The resolution factor for Zidovudine from the nearest resolving solvent peak was > 3 in all samples. The placebo shows no detector response near retention times of 4.695 min, while the Zidovudine standard displayed good resolute peak [Figure 4] and no interference from excipients present in the formulation [Figure 5]. Both these characters show the specific nature of the method. The peak purity curve and data [Figure 6 & Table 14] of Zidovudine shows that no other excipients are co-eluted with the drug and the peak is pure in nature.

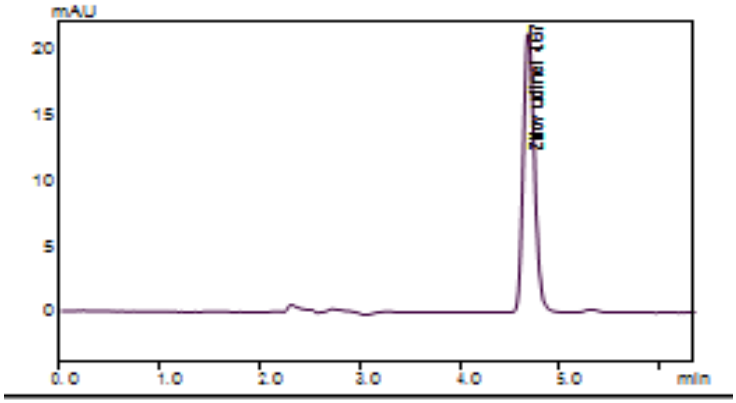


Figure 4: Chromatogram showing peak of Zidovudine at 266 nm in bulk drug

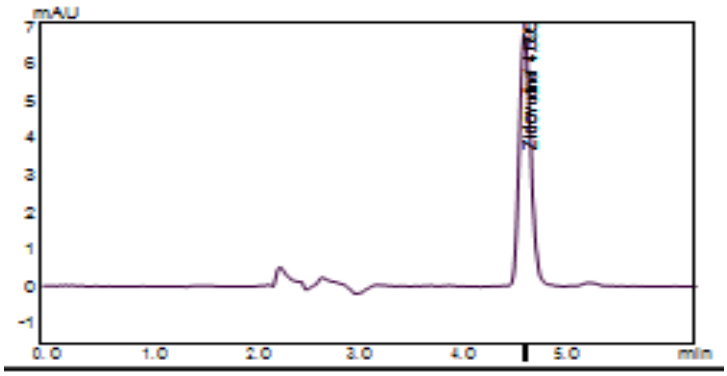


Figure 5: Chromatogram showing peak of Zidovudine at 266 nm in formulation

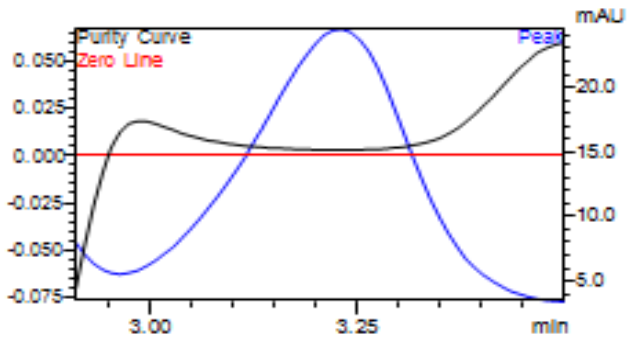


Figure 6: Peak Purity Plot of Zidovudine with purity 0.99974

Table 14: Peak purity data for determination of Zidovudine

Drug	Peak purity Index	Single point Threshold	Min peak purity
Zidovudine	0.99974	0.99921	4824

➢ **System suitability**

As system suitability test was an integral part of chromatographic method development and were used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Zidovudine were evaluated. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values. The obtained parameters are given in table 15 and they are found to be in concordance to the acceptance criteria.

Table 15: System suitability parameters for Zidovudine by RP-HPLC method

Parameter	Value for Zidovudine
Retention time (Minutes)	4.69
Resolution (Rs)	3.25
Theoretical plates (Tp)	7563
Tailing factor (Tf)	0.93
Asymmetric factor (Af)	0.57
Capacity factor (K')	3.01

➢ **Solution stability**

The % CV of the assay of Zidovudine during solution stability experiments were within 2 %. No significant changes were observed in the content of standard drug solution during solution stability and mobile phase stability experiments when performed using the method. The solution stability and mobile phase stability experiment data confirms that the sample and standard in solvent and mobile phases used during assay determination were stable for at least 24 hours. The results of solution stability data are shown in table 16.

Table 16: Solution stability data for Zidovudine

	Peak Area Zidovudine 4 µg/mL		
Time (Hr)	Standard	Sample	% sample stability
00	81837	81456	100
06	81634	81438	99.98
12	81582	81411	99.94
18	81531	81389	99.92
24	81287	81376	99.90

- **Comparison of developed method with the official method**

The assay results in terms of AUC calculated as assay % were compared with each other for both the developed method and the standard method after 100% spiking with a known concentration of the standard drug solution. Six replicate readings were taken for each method. The results were compared by using t-test. At 5% probability the tabulated value was 2.57. The calculated value was obtained as 1.3657. Hence it is deduced that there is no significant difference between the accuracy of the two methods.

4. CONCLUSION

A validated RP-HPLC analytical method has been developed for estimation of Zidovudine in bulk and in formulation. The proposed method is very fast, simple, accurate, precise, specific, and has ability to separate drug from excipients. The method is suitable for routine analysis of Zidovudine in capsule formulation. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments. Also the method requires no sample pretreatment and is quite economical for routine analyses. The proposed RP-HPLC method developed meets the system suitability criteria, peak integrity and resolution for the drug. Detection and quantification limits achieved describe that the method is quite sensitive. High recoveries and acceptable % CV values confirm that the proposed method is accurate and precise. The analytical results demonstrate the ability of the developed method to assay the drug in the presence of its excipients. Also the data for precision show that developed method is precise. Assay results found from the study show that the method can be successfully applied for the estimation of Zidovudine in capsule formulation. Through statistical techniques it is concluded that there is no significant difference between the accuracy of the proposed method and the official method. Hence the proposed method is recommended for routine analysis of Zidovudine in bulk and in capsule formulation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

There is no any conflict of interest by author.

REFERENCES

1. N.J. The Merck index. An encyclopedia of chemicals, drugs and biological: Published by Merck research laboratories, Merck & Co., Inc. 14th Edition, Whitehouse Station, USA; 2006.
2. Validation of Analytical procedures: Text and Methodology Q2(R1), International Conference on Harmonization: ICH Harmonised Tripartite Guideline; International Conference on Harmonisation of Technical requirements for registration of pharmaceuticals for human use; Current step 4 version.2005.
3. Indian Pharmacopoeia. Published by the controller of publications Vol-II and Vol III, Delhi: India; 2010.
4. MD. The United States Pharmacopoeia, US Pharmacopoeial Convention 26th Ed. Rockville, 1151.
5. British Pharmacopoeia. Her Majesty's Stationary Office. Vol. II, London; 2003.
6. Sanne, Ian* Piliero, Peter†, Squires, Kathleen‡ Thiry, Alexandra§ Schnittman, Steven§. AI424-007 Clinical Trial Group; A Dose-Ranging, Safety, and Efficacy Comparative Trial of Atazanavir at Three Doses in Combination with Didanosine and Stavudine in Antiretroviral: Journal of Acquired Immune Deficiency Syndromes. 2003; Volume 32(1): 18-29.
7. Coakley Eoin P, Gillis, Jacqueline M., Hammer Scott M. Phenotypic and genotypic resistance patterns of HIV-1 isolates derived from individuals treated with Didanosine and Stavudine: AIDS Journal of Acquired Immune Deficiency Syndromes. 2000; 14(2): F9-F15.
8. Raffi F et al.. Department of Infectious Diseases, University Hospital Nantes, Hydroxyurea in combination with Didanosine and Stavudine in antiretroviral-experienced HIV-infected subjects with a review of the literature: Int J STD AIDS. 2003; 14(5):350-5.
9. Rossero R et al. Department of Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, USA, Combination Therapy With Stavudine (d4T) Plus Didanosine (ddI) in Children With Human Immunodeficiency Virus Infection: PEDIATRICS, 1999; 103(5): e62.
10. Lafeuillade A et al. Department of Infectious Diseases, General Hospital, Toulon, France. Effects of a combination of Zidovudine, Didanosine, and Lamivudine on primary human immunodeficiency virus type 1 infection: J Infect Dis. 1997;175(5):1051-5.
11. Shafer RW et al. Stanford University Medical Center, CA 94305; Combination therapy with Zidovudine and Didanosine selects for drug-resistant human immunodeficiency virus type 1

strains with unique patterns of pol gene mutations: J Infect Dis. 1994;169(4):722-9.

12. Robert N. Husson MD et al. Zidovudine and Didanosine Combination Therapy in Children with Human Immunodeficiency Virus Infection: Pediatrics.1994; 93(2) 316-322.
13. Hedaya MA, Sawchuk RJ. A sensitive and specific HPLC method for the determination of Zidovudine and Zidovudine glucouronide in plasma and urine: Int Conf AIDS**.** 1989; 5: 278.
14. M Rubiana, Mainardes and D Maria Palmira. Gremião. Reversed phase HPLC determination of Zidovudine in rat plasma and its pharmacokinetics after a single intranasal dose administration: Biological Research. 2009; 42: 357-364.
15. Alnouti Y, Lewis SR, White CA, Bartlett MG. Simultaneous determination of Zidovudine and Lamivudine from rat tissues by liquid chromatography / tandem mass spectrometry: Rapid Commun Mass Spectrom. 2005; 19(4):503-8.
16. Fan B, Stewart JT. Determination of Zidovudine, Lamivudine and Nevirapine in human plasma using solid-phase extraction procedure: J Pharm Biomed Anal. 2002; 28(5):903-8.
17. Pai N, Desai AD. Simultaneous reverse phase HPLC estimation of some antiretroviral drugs from tablets (Lamivudine, Zidovudine and Nevirapine): Indian Journal of Pharmaceutical Sciences. 2007; 69:118-20.
18. M. S Palled, P. M. N Rajesh, M Chatter, A. R Bhat. Reverse phase high performance liquid chromatographic determination of Zidovudine and Lamivudine in tablet dosage forms: Indian Journal of Pharmaceutical Sciences 2005; 67(1):110-112.
19. G Ramachandran, AK Hemanthkumar, V Kumaraswami, S Swaminathan. A simple and rapid liquid chromatography method for simultaneous determination of Zidovudine and Nevirapine in plasma: Journal of Chromatography B Analytical Technology Biomedical Life Sciences. 2006; 843(2):339-44.
20. C.P.W.G.M. Verweij-van Wissen, R.E. Aarnoutse and D.M. Burger. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors Lamivudine, Didanosine, Stavudine, Zidovudine and Abacavir in human plasma by reversed phase high performance liquid chromatography: Journal of Chromatography B. 2005; 816(1-2):121-129.