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Research Article

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF ZIDOVUDINE

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ABSTRACT

This work describes a new, fully validated, simple, rapid, selective, and sensitive HPLC method with UV detection for the direct determination of zidovudine in pharmaceutical dosage forms, raw materials, spiked human serum, and drug dissolution studies without any time-consuming extraction or evaporation steps prior to drug assay. The mobile phase employed was methanol: acetonitrile (40:60v / v / v). The samples of 20 µL were injected onto a Zodiac100-5 C18) 250×4.6mm column. The flow rate was 1.0 mL min^{-1}. The retention times were 2.51 min Zidovudine at 2.51min. The samples were detected at 270 nm. The assay was linear in the concentration range 0.1-0.6 µgmL^{-1} (r = 0.995) with a slope of 188680; intercept of a 4018.33 and the limit of detection was limit of detection was 0.062 µg mL^{-1}. It was successfully applied to the analysis of pharmaceutical preparations without any interference by the excipients and endogenous substances. Moreover, the method can be used for the determination of Zidovudine for monitoring its concentration for in vitro dissolution studies.

Keywords: Zidovudine, method development, validation.

INTRODUCTION

Zidovudine (INN) or azidothymidine (AZT) (also called ZDV) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV / AIDS. It is an analog of thymidine. AZT was the first approved treatment for HIV, sold under the names Retrovir and Retrovis. AZT use was a major breakthrough in AIDS therapy in the 1990s that significantly altered the course of the illness and helped destroy the notion that HIV / AIDS was a death sentence. Zidovudine is chemically 1-[(2R,4S,5S)-4azido-5(hydroxymethyl) tetrahydrofuran-2-yl]-5-methylprimidine-2, 4 (1H, 3H0-dione and used as an antiretroviral activity. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances.

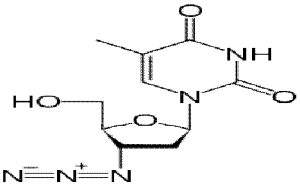


Fig. 1: Chemical Structure of Zidovudine

The proposed method describes the development and validation of a stability indicating method for the assay of Zidovudine in tablets by HPLC and the procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

EXPERIMENTAL

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Zodiac100-5 C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample, PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance.

Reagents and Materials

Working standard of Zidovudine was obtained from well reputed research laboratories. HPLC grade water, methanol, Acetonitrile was purchased from E. Merck (Mumbai, India).

Determination of wavelength of maximum absorbance

The standard solutions of Zidovudine were scanned in the range of 200 -400 nm against mobile phase as a blank. Zidovudine showed maximum absorbance at 270 nm. So the wavelength selected for the determination of Zidovudine was 270 nm.

Chromatographic conditions

The Zodiac C18 column was used at ambient temperature. The mobile phase consisted of Mobile phase: methanol: acetonitrile (40:60 v/v) and the flow rate was maintained at 1 ml/min. The mobile phase was passed through nylon
0.45µm– 47mm membrane filters and degassed before use. The elution was monitored with UV detector at 270 nm, and the injection volume was 20µL.
HPLC method depends upon the nature of the sample (ionic or ignitable or neutral molecule), its molecular weight and solubility. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

Standard preparation

Preparation of DONE Standard Stock Solutions (100µg/ml)

Accurately weighed 25mg of Zidovudine transferred to a 25ml volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard solution of 1000µg/ml. This solution (1ml) was further diluted to 10 ml with mobile phase to obtain a working standard stock solution of 100µg/ml for the RP- HPLC method.

Method development

Optimization of the chromatographic condition

Several mobile phases were tried to resolve Zidovudine but the resolution was not satisfactory. So modification was made in the above mobile phase. Finally the system containing methanol:acetonitrile (40:60 v/v) as the mobile phase at a flow rate of 1.0ml/min was found to be satisfactory and gave well resolved peak for Zidovudine . The retention time for Zidovudine was 2.52 min. For the selection of detection wavelength, the spectrum of 10 ppm Zidovudine revealed that, at 270 nm the drug possesses significant absorbance. So considering above fact, 270 nm was selected as a detection wavelength for estimation of Zidovudine using HPLC. Complete resolution of the peaks with clear baseline separation was obtained. The system suitability test parameters are shown in Table 1.

Method Validation

Linearity and range

Linearity was demonstrated by analyzing six different concentrations of active compound. Accurately measured standard working solutions of Zidovudine 0.1, 0.2, 0.3, 0.4, 0.5, 0.6µg/ml were prepared in 10ml volumetric flasks and diluted to the mark with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs. concentrations Zidovudine. Coefficient of

correlation was 0.988. Validation parameters shown in Table 2.

Accuracy (recovery)

The accuracy of the method was determined by calculating recoveries of Zidovudine by the standard addition method. Known amounts of standard solutions of Zidovudine (50%) were added to pre quantified sample solutions of tablets. The amounts of Zidovudine were determined by applying these values to the regression equation of the calibration curve. Recovery values can shown in Table:3.

Method precision (repeatability)

The precision of the method was checked by repeatedly injecting (*n* = 6) solutions of Zidovudine (20µg/ml) for the RP-HPLC method. The accuracy of the method was evaluated by determination of the recovery of Zidovudine on two days at six levels concentration. Tablets and capsules sample solutions were spiked with Zidovudine standard solution, corresponding to 75 to 125% of the nominal analytical concentration (30µg/ml). The results showed good recoveries ranging from 98.77 to 101.45%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.

Limit of Detection (LOD)

The sample was dissolved by using Mobile Phase and injected until peak was diapered. After 0.053µg/ml dilution Peak was not clearly observed. So it confirms that 0.062µg/ml limit of Detection.

RESULT AND DISCUSSIONS

UV spectrum of recorded from which 260--280 nm was selected as wavelength. Flow rate of 1ml/min was selected. Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase.

Mixture of 40% methanol+ 60% acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of Zidovudine was thoroughly investigated. The concentration of the methanol, acetonitrile was optimized to give symmetric peak with short run time. The retention time was found to be 2.51. Zidovudine shown linearity in the range of 10-60µg/ml, and the co-efficient was found to be 0.995. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

System Suitability Parameter	Zidovudine
Retention Time	2.51min
Tailing factor	1.16
Theoretical plate	4044

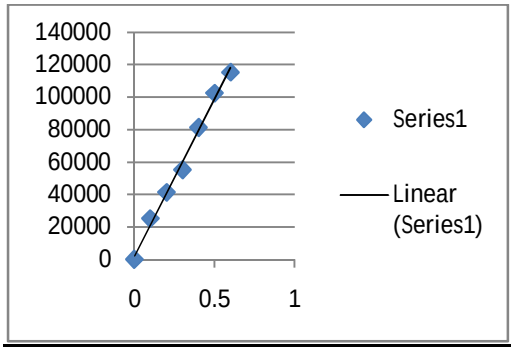


Fig. 2: Graph linearity

Table 2: Optical and Regression characteristics and validation parameters of HPLC method

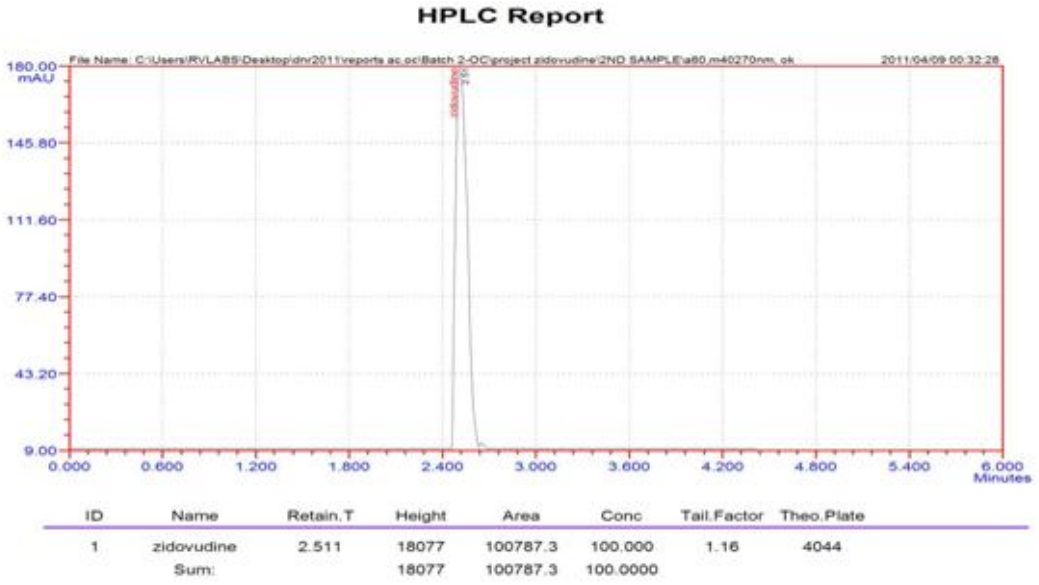
Parameters	Concentration (µg/ml)
Calibration range	0.1-0.6
Detection limit	0.154
Quantitation limit	0.825
Slope	188680.3
Intercept	4018.33
Correlation coefficient	0.995
Intraday RSD, %	101.2
Interday RSD, %	98.7

Table 3: Data of recovery study for Zidovudine by HPLC method

Amount taken (µg/ml	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
10	2	12.10	98.5
10	4	13.22	99.65
10	6	15.65	102.5

CONCLUSION

A RP-HPLC method has been developed for the determination of Zidovudine. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Zidovudine. The results of the study reveal that the proposed RP-HPLC method for the estimation of Zidovudine is simple and accurate in bulk and pharmaceutical dosage forms.



REFERENCES

1. Hedaya MA and Sawchuk RJ. International Conference on AIDS. University of Minnesota, Minneapolis, Minnesota, USA. 1989.
2. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=35370.
3. http://whqlibdoc.who.int/hq/2005/a87017_eng.pdf.
4. Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. Antiviral research. 2009;85(1):1–2.
5. Horwitz JP, Chua J and Noel MJ. The monomesylates of 1-(2-deoxy-bd-lyxofuranosyl) thymines. Org Chem Ser Monogr. 1964;29(7):2076–9.
6. A recent book provides a comprehensive treatment of the theory of high-performance gradient chromatography: Lloyd R. Snyder and John W. Dolan (2006). High-Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model. Wiley Interscience. ISBN 0471706469.
7. Xiang Y, Liu Y and Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography A. 2006;1104(1–2):198–202.
8. Horváth CS, Preiss BA and Lipsky SR. Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. Analytical Chemistry. 1967;39(12):1422–1428.
9. International Conference on Harmonization. "Q2A: Text on Validation of Analytical Procedures," Federal Register. 1995;60(40):11260–11262.
10. International Conference on Harmonization. "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register. 1997;62(96):27463–27467.