



UV Visible Spectrophotometric Estimation of Zanamvir

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ABSTRACT

A low cost precise spectrophotometric method has been developed for the estimation of Zanamvir in bulk and tablet dosage form. Zanamvir shows maximum absorbance at 260nm in presence of Double Distilled Water and phosphate buffer of pH 7.4. The Beer's law is obeyed in the concentration range of 2-10 µg/ mL for this drug. The graph of the drug shows a straight line with correlation coefficient of 0.9999. The assay method of the drug was validated by accuracy and precision of the proposed method. The results are validated as per the directions of International conference on Harmonization.

Keywords: UV Spectrophotometry; Zanamvir; Double distilled water; Beer's law

INTRODUCTION

Zanamivir is a first neuraminidase inhibitor used in the treatment and prophylaxis of influenza caused by influenza A virus and influenza B virus. The chemical name of zanamivir is (2R,3R,4S)-4-guanidino-3-(prop-1-en-2-ylamino)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid[1]. It has a molecular formula of C₁₂H₂₀N₄O₇ and a molecular weight of 332.31g/mol, white crystalline powder and soluble in water and alcohol. Literature survey of Zanamivir reveals that only one spectrophotometric method have been reported by using Acetonitrile & water as solvent by Nevin E.R.K[2]. Therefore, an attempt was made to develop a low cost precise, accurate spectrophotometric method for the estimation of Zanamivir in bulk and tablet dosage form.

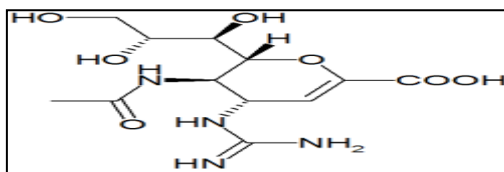


Figure 1: The chemical structure of Zanamvir

EXPERIMENTAL SECTION

Chemicals

All chemicals are AR grade, Double distilled Water and phosphate buffer of pH 7.4 is used throughout the analysis and purchased from Merck India Ltd, Mumbai. Pharmaceutical formulation of Zanamvir was supplied by Lupin India Ltd, Hyderabad. Commercially available tablets namely Rebetol and Virazol procured from Medwin pharmacy, Hyderabad

Instrument

The absorbance of the drug Zanamvir were carried out by using shimadzu company model 1700 UV-visible double beam spectrophotometer with 1 cm matched quartz cell, spectral band width is 1 nm, supported by UV win 5.0 software.

Selection of solvent

Double distilled water and phosphate buffer of pH 7.4 [4] are used throughout the analysis.

Selection of method and wave length

UV scan range of 200 nm to 400 nm was selected for the proposed method of Zanamvir . The wavelength corresponding to maximum absorbance was found at 260 nm and calibration curve was taken at 260 nm. The intercept of calibration line of the drug was determined by Linear regression Analysis

Preparation of standard solutions of Zanamvir

The 10 mg of standard (pure) drug of Zanamvir is weighed accurately and dissolved in 100 ml Double distilled Water then transferred into 100 ml volumetric flasks to prepare 1000 µg/ mL [3] stock solution of the drug. Then different aliquots of 2- 10 µg/mL were taken in eight 10 ml volumetric flasks .To each flask 2mL of phosphate buffer of pH 7.4 solution is added, then all stock solutions of the drug were scanned in the UV scan range of lambda max (λ_{max}) 200 nm to 400 nm to determine maximum absorbance for this method .The calibration curve was plotted in the concentration range of 2-10 µg/ mL. The wavelength corresponding to maximum absorbance of Zanamvir measured at 260nm against Double distilled Water as blank.

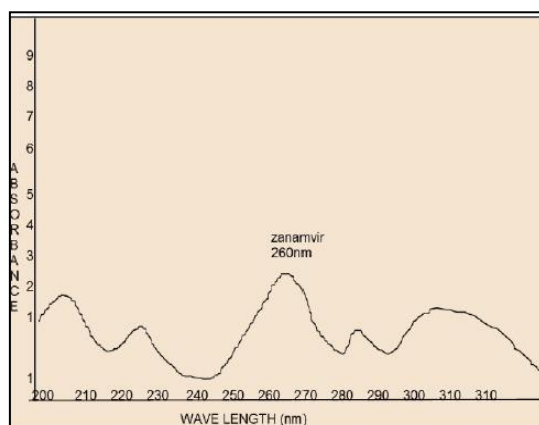
Preparation of sample solutions of Zanamvir

For the analysis of Zanamvir, two commercial brands namely Rebetol(10mg) and Virazol (10mg),tablets were purchased from Medwin pharmacy ,Hyderabad. Twenty tablets of the drug was weighed accurately and powdered, and then 100 mg of the drug in powdered form dissolved in 70 ml of Double distilled Water and sonicated for few minutes and filtered by using whatmann filter paper No.42. The filtrate having 10µg/mL concentration and further diluted with Double distilled Water then taken in a eight 10 ml volumetric flasks. To each 10 ml flask 2mL of phosphate buffer of pH 7.4 solution is added. Then absorbance of Zanamvir measured at 260 nm against Double distilled Water as blank.

Determination of λ_{max}

UV scan range of 200 nm to 400 nm was selected to determine maximum absorbance by using 10 µg/ml solution of the drug, the wave length corresponding to maximum absorbance was found at 260 nm for this drug. The spectrophotometric spectrum of Zanamvir is shown in figure 2.

Figure 2: UV Spectrum of Zanamvir

**Preparation of calibration curve**

On the basis of experimental results, calibration curve is plotted and shown in figure 3 in the concentration range of 2-10µg/ mL of eight standard solutions of Zanamvir in Double distilled Water as blank. UV scan range of 200 nm to 400 nm was selected to determine maximum absorbance of the drug. In this method the wavelength corresponding to maximum absorbance was found at 260 nm.

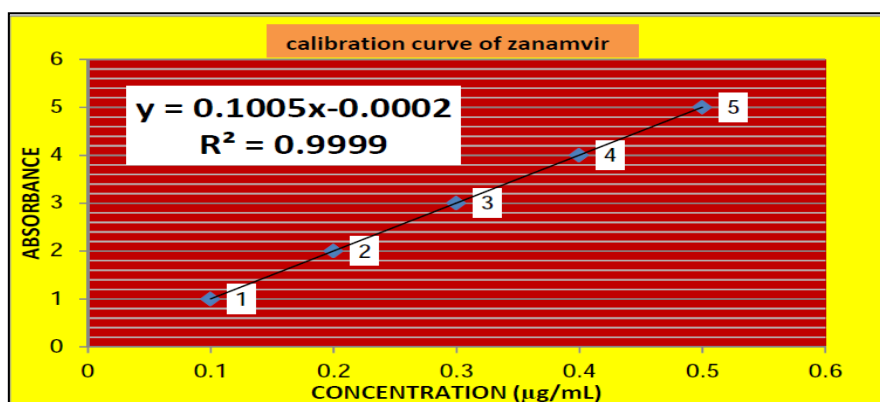
Validation of method [5]

The spectrophotometric estimation of Zanamvir is validated as per the directions of International conference on Harmonization to determine linearity, precision, accuracy, LOD and LOQ of the proposed method.

Linearity and range

Standard stock solution of Zanamvir in appropriate dilution were assayed as per the proposed method According to Beer's –Lambert's law the concentration range of Zanamvir was found to be 2-10 µg/ mL ,So that the calibration curve in the figure 3 is linear in the given concentration range.

Figure 3: Calibration curve of Zanamvir



Precision

The precision of the proposed method of Zanamvir was estimated by using drug concentration of Zanamvir were analyzed six times in a day (intra-day precision) and six continuous days (inter-day precision). Data is given in the table 1

Accuracy

The Accuracy of the proposed method of Zanamvir was estimated by using standard addition method .This process is carried out by adding different amounts namely 80% ,100% and 120% of the pure sample of the drug to the pre-analysed formulation. Accuracy data of the drug is shown in the table 1.

Table 1: Precision and accuracy of Zanamvir

S No	Name of the sample	Labeled amount (mg/capsule)	Amount found* (mg)	Precision	
				Inter day	Intra day
1	Rebetol	10	9.8	0.0072	0.0082
2	Virazol	10	9.9	0.0094	0.0081

*average of 6 determinations

LOD and LOQ

LOD is Limit of Detection and LOQ is Limit of Quantitation. The LOD and LOQ of Zanamvir were determined (Table 2) by using standard deviation of the response and slope approach as per the directions of International Conference on Harmonization (ICH) guidelines. The limits of detection (LOD) is calculated by using the equation $LOD = \frac{3s}{k}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) The limits of quantitation (LOQ), is calculated by using the equation $LOQ = \frac{10S}{K}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean).

Table 2: Optical Parameters of Zanamvir

S No	Parameter	Zanamvir
1	λMax (nm)	260nm
2	Beer's Law Limit (µg/mL)	2-10
3	Correlation Coefficient (r2)	0.9999
4	Regression Equation (Y=a+bc)	0.01005×0.0002
5	Intercept (a)	0.0002
6	Slope (c)	0.1005
7	SD	2.7386
8	Mean	6
9	Variance	7.5
10	LOD (%)	0.0059
11	LOQ (%)	0.0199

Recovery studies of Zanamvir

Recovery of Zanamvir was performed to know the accuracy of the proposed method. This process is done by adding a known quantity of pure drug to a pre-analysed sample. The result of analysis of the drug is notified in the Table 3.

Table 3: Recovery Analysis of Marketed Formulation of Zanamvir

S No	Name of the sample	Labeled amount (mg/capsule)	% Level	Amount found* (mg)	% Recovery
1	Rebetol	10	120	9.8	99
2	Virazol	10	180	9.9	99

RESULTS AND DISCUSSION

The U.V Spectrum of standard stock solutions of Zanamvir shows absorption maximum at 260 nm, then the calibration curve is obtained by plotting a graph of absorbance verses concentration, the Beer –lamberts' law was verified from the data of calibration curve of the drug under investigation. The calibration curve of the drug is shown in the figure 3. The linearity was observed between 2-10 µg/ mL for Zanamvir. The graph of this drug shows a straight line with correlation coefficient of 0 .9999. The assay method of the drug was validated by the accuracy and precision of the proposed method shown in table 1. The % recovery of 99-99 shows accuracy of the proposed method. The validated optical, statistical parameters, LOD and LOQ data of Zanamvir has been given in table 2.

CONCLUSION

The developed method was found to be simple, sensitive, accurate and can be used for routine quality control and analysis of Zanamvir in bulk as well as in pharmaceutical dosage form.

ACKNOWLEDGMENT

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