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Spectrometric Method Development for the Estimation of Cidofovir Dihydrate in Bulk and Pharmaceutical Formulation

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Abstract: ***A simple, specific and rapid difference spectroscopic method has been developed for the estimation of Cidofovir dihydrate in bulk and Pharmaceutical formulation. The proposed method was carried out by measuring the difference absorbance of Cidofovir dihydrate in two different conditions containing three different forms of drug generated by neutral (solvent), acidic (solvent) and basic (solvent) medium. The measurements of difference absorbance were carried out at 270 nm for two different conditions. The calibration curves were linear in the concentration range of 10- 60µg/ml. The proposed method was validated as per ICH validation guideline Q2 (R1) for accuracy, robustness, LOD, LOQ etc. The method was found to be accurate, precise, robust and sensitive hence it can be applied in routine analysis of Cidofovir dihydrate in bulk and Pharmaceutical formulation in its quality control.***

Keywords: Cidofovir, Infra-red Spectroscopy, Potassium Bromide, Acetone.

1. Introduction

Cidofovir dihydrate, a nucleotide analogue antiviral drug, is used for the treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). Chemically it is ({[(S)-1-(4-amino-2-oxo-1,2- dihydropyrimidine-1-yl)-3-hydroxypropan-2- yl] phosphoric acid dihydrate.

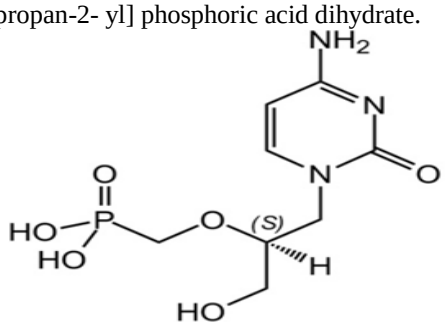

Figure 1: Chemical Structure of Cidofovir ({[(S)-1-(4-amino-2-oxo-1,2- dihydropyrimidine-1-yl)-3-hydroxypropan-2- yl] phosphoric acid dihydrate.

The structural formula of Cidofovir is: The extensive literature survey revealed that analytical methods like Infra-red Spectrophotometry have been reported for the estimation of CDV in bulk and Pharmaceutical dosage forms. But no any simple difference spectrophotometric method was available for CDV estimation in bulk and Pharmaceutical dosage forms. So it was thought of interest to develop and validate a rapid, cost effective and precise difference spectrophotometric method for the determination of CDV in bulk and Pharmaceutical formulation.

2. Material and Methods

2.1 Instruments used

•The IR used was model SHIMADZU.
•SHIMADZU Electronic balance, Japan.

2.2 Reagents and chemicals

Cidofovir used as an internal standard. EmcurePharma Ltd. Pune)
Potassium Bromide, Acetone. (Shivaji Scientific Supplier, Pune)
All other chemicals were of analytical grade and used without any further purification.

3. Experimental

3.1 Apparatus

Volumetric Flask, Beaker, Pipette, Funnel.

3.2 Spectroscopy Measurements:

IR spectra of reference and sample mixture were recorded in pellets of 150 mg. The absorbance values obtained in the spectra were obtained at 1757-1671 cm-1 for quantification of bulk drug 300 mg. The spectroscopy measurements were recorded by using potassium bromide as a blank.

3.3 Preparation of Pellets:

Stock and Working Standard Mixture:
Stock standard mixture containing Cidofovir at a concentration of 1:10 was prepared by accurately weighing 70 mg of Cidofovir reference substance and 630 mg of potassium bromide. Working standard mixture were prepared immediately before use by suitable dilutions of the corresponding stock mixtures to appropriate concentration levels by using potassium bromide as diluent.

3.4 Analytical Method Validation Parameters

3.4.1 System Suitability

To assess system suitability of the method, the repeatability, theoretical plates, tailing factor and retention time of six replicate injections of standard Cidofovirof concentration 100 µg/ml were used and the %RSD values were calculated in each case.

3.4.2 Linearity

The linearity was analyzed through the standard curves ranging from 10 to 60 µg/ml by diluting appropriate amounts of Cidofovir stock solution (1000 µg/ml) with methanol and prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

3.5 Specificity

The specificity of the developed IR method for the determination of Cidofovir in bulk drug.

3.6 Precision

Precision of the method was determined by repeatability (intraday precision) and intermediate precision (Interday precision) of both standard and sample solutions. Precision was determined in six replicates of Cidofovir standard solution (100 µg/ml). The results were expressed as %RSD of the measurements.

3.7 Sensitivity

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined using calibration curve method according to ICH Q2 (R1) recommendations. The LOD (k=3.3) and LOQ (k=10) of the proposed method were calculated using the following equation: $A=k\sigma/S$,(1) where A is LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

3.8 Ruggedness

Ruggedness of the current method was determined by analyzing six assay standard solutions of Cidofovir having concentration of 100 µg/ml by two analysts in the same laboratory to check the reproducibility of the test result. The % recovery and standard deviation were calculated.

3.9 Robustness

To determine the robustness of the current method, the effect of flow rate was studied at 0.1 and 2ml /min instead of 1.0 mLmin−1. The effect of column temperature was studied at 25 and 35°C instead of 30°C. The effect of mobile phase composition was assessed at (water: methanol= 20:80,v/v) and (water:methanol = 40:60,v/v) instead of (water:methanol = 30:70,v/v). The %RSD of robustness testing under these conditions was calculated in all cases.

4. Results and Discussion

Method Validation

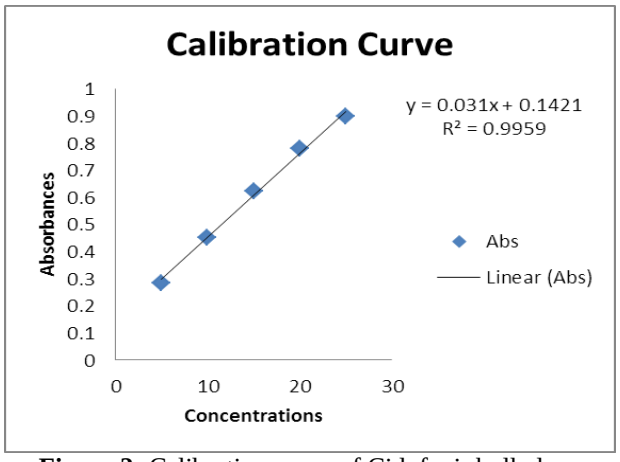

Figure 2: Calibration curve of Cidofovir bulk drug

4.1. Linearity

The regression equation for Cidofovir was found y=0.031x + 0.1421 by plotting absorbance (y) versus the concentration (x) studied from 5 to 25µg/ml, and the correlation coefficient (R2=0.995) was highly significant. The validity of the assay was verified by means of the ANOVA. According to it, there is linear regression and there is no deviation from linearity (P<0.05).

Table 1: Linearity of Impurity of Cidofovir

Sr. No.	Conc. (µg/ml.)	Abs.
1	5	0.286
2	10	0.452
3	15	0.622
4	20	0.779
5	25	0.898

4.2 Precision

The values of %RSD for intraday and Interday variation are given in Table. In both cases, %RSD values were found well within 2% limit, indicating that the current method is repeatable.

Table 2: Intraday precision of Cidofovir

Sr. No.	Concentration (µg/ml)	Absorbance at 270nm(after4hr)	Mean	S.D.	%RSD
1.	40	1.02602	1.02648	0.01185	1.15494
2.	40	1.03201			
3.	40	1.01210			
4.	40	1.04621			
5.	40	1.01798			
6.	40	1.02456			

Table 3: Interday precision of Cidofovir

Sr. No.	Concentration (µg/ml)	Absorbance at 270nm(after 24 hr.)	Mean	S.D.	%RSD
1.	40	1.02507	1.02486	0.00860	0.83940
2.	40	1.03110			
3.	40	1.01476			
4.	40	1.03668			
5.	40	1.01549			
6.	40	1.02610			

4.3 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 x σ / S and LOQ = 10 x σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.0968 µg/ml and 0.2904µg/ml respectively.

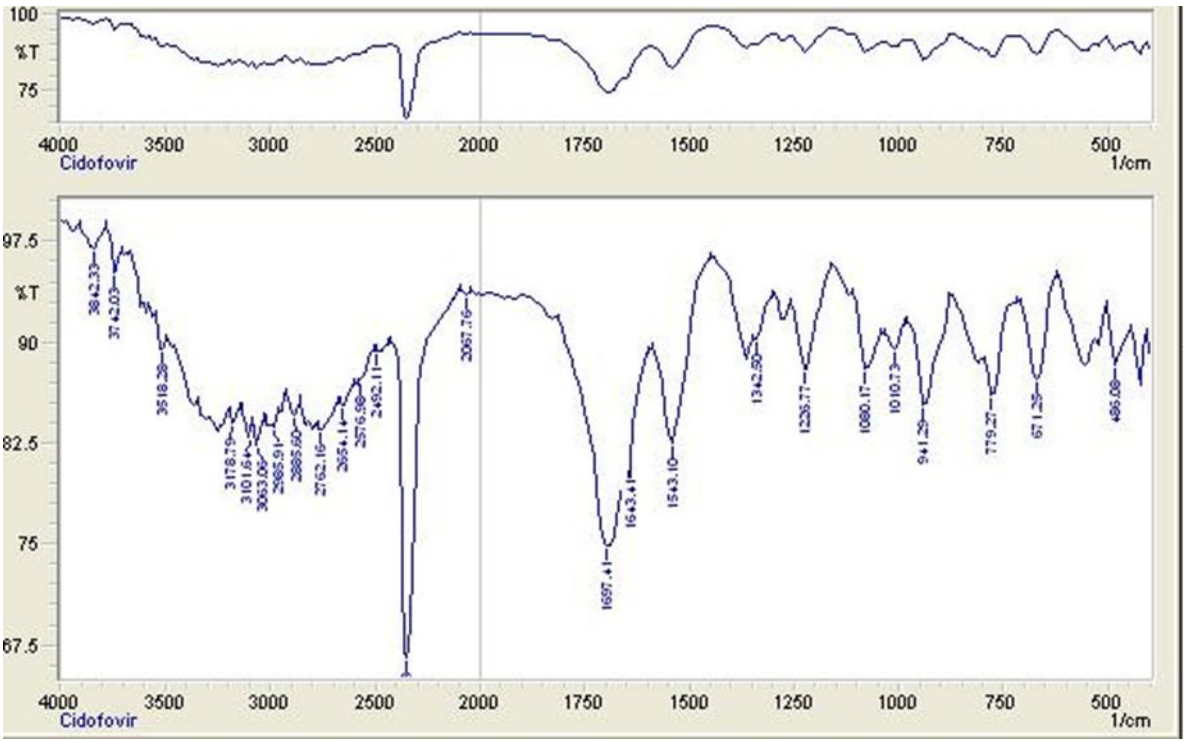

Figure 3: IR spectra of Cidofovir

5. Conclusions

In this work, an analytical IR spectroscopy method was successful developed for quantitative determination of Cidofovir in tablets. Its advantages over other existing method are its simplicity, inexpensive conditions and it does not use polluting reagents. The results indicated that the IR spectroscopy method presents linearity, selectivity, accuracy, precision, robustness and adequate detection and quantification limits. Therefore, the validated method can be easily applied in routine analysis of Cidofovir.

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