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RESEARCH ARTICLE

Development and Validation of Difference Spectrometric Method 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 290 nm for two different conditions. The calibration curves were linear in the concentration range of 5- 25µg/ml. The proposed method was validated as per ICH validation guideline $Q_2(R_1)$ 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

Difference spectrometry, Cidofovir, Validation

INTRODUCTION

Cidofovir dihydrate, a neucleotide analogue anti-viral drug, is used for the treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS)[1]. Chemically it is ({[(S)-1-(4-amino-2-oxo-1,2-dihydropyrimidine-1-yl)-3-hydroxypropan-2-yl]phosphonic acid dihydrate[1]. It was approved by USFDA in May 1996[2]. It is also used for the management of acyclovir- resistant herpes simplex virus infections in immuno compromised patients. Intravenous CDV has been used for treating acyclovir- resistant mucocutaneous HSV infection, adenovirus disease in transplant recipients and progressive multifocal leuko-encephalopathy extensive molluscum contagosum in HIV patients.

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The extensive literature survey revealed that analytical methods like HPLC[3], Spectrophotometry[4] have been reported for the estimation of CDV in bulk and Pharmaceutical dosage forms as well as HPLC- MS[5] and HILIC[6] for estimation in plasma samples. 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.

MATERIAL AND METHODS

Cidofovir dihydrate (CDV) was procured from Emcure Pharma Pvt. Ltd., Pune, Maharashtra.

The Cidofovir injection (75mg/ml) was procured from the market.

Preparation of Standard Stock Solution

Accurately weighed (25 mg) CDV was transferred to a 25 ml volumetric flask, dissolved in and diluted up to the mark with distilled water to obtain a standard stock solution (1000µg/ml). An aliquot (1ml) was transferred to 10 ml volumetric flask and diluted up to the mark with water to obtain the working standard solution (100µg/ml).

Preparation of Hydrochloric acid (0.1N)

Accurately measured 0.85 ml concentrated hydrochloric acid (36%) was transferred to 100 ml volumetric flask and diluted up to the mark with distilled water.

Preparation of Sodium hydroxide (0.1N)

Accurately weighed (0.4 gm) sodium hydroxide was transferred in to 100ml volumetric flask, dissolved in about 60 ml distilled water and diluted up to the mark with distilled water.

Preparation of Sample Solution

For analysis of the CDV in injection, the appropriate volume of injection (1ml) having strength of 75mg/ml (75000µg/ml) CDV was transferred to the 100ml volumetric flask and diluted up to the mark with water to obtain the working standard solution (750µg/ml). An aliquot (0.2 ml) was taken in triplicate 10 ml volumetric flasks, and volumes were made up to the mark with water, 0.1N HCl and 0.1N NaOH respectively to prepare test solution containing 15µg/ml. The above solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra at the below mentioned conditions.

Selection of Wavelength for Determination

- Wavelength was determined for two conditions

Condition 1: Water (Ref.) - HCl (Sample)

Different aliquots of appropriate volume were taken in a series of 10ml volumetric flasks from the working standard solution and diluted up to the mark with distilled water and 0.1N HCl separately to prepare the concentration range of 5- 25µg/ml. The solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra by keeping the acidic form (i.e. CDV in 0.1N HCl) in sample cell and neutral form (i.e. CDV in water) in reference cell using 0.1N HCl (in sample cell) and water (in reference cell) as blank. The maximum difference absorbance was observed at 290 nm which was selected for analysis.

Condition 2: NaOH (Ref.) - HCl (Sample)

Different aliquots of appropriate volume were taken in a series of 10ml volumetric flasks from the working standard solution and diluted up to the mark with 0.1N NaOH and 0.1N HCl separately to prepare the concentration range of 5- 25µg/ml. The solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra by keeping the acidic form (i.e. CDV in 0.1N HCl) in sample cell and neutral form (i.e. CDV in 0.1N NaOH) in reference cell using 0.1N HCl (in sample cell) and water (in reference cell) as blank. The maximum difference absorbance was observed at 290 nm which was selected for analysis.

RESULTS AND DISCUSSION

The calibration standards of CDV were prepared in concentration range of 5- 25µg/ml and analyzed spectrophotometrically to generate calibration curve of difference absorbance vs. concentration. A regression coefficient was obtained above 0.994 in both the condition.

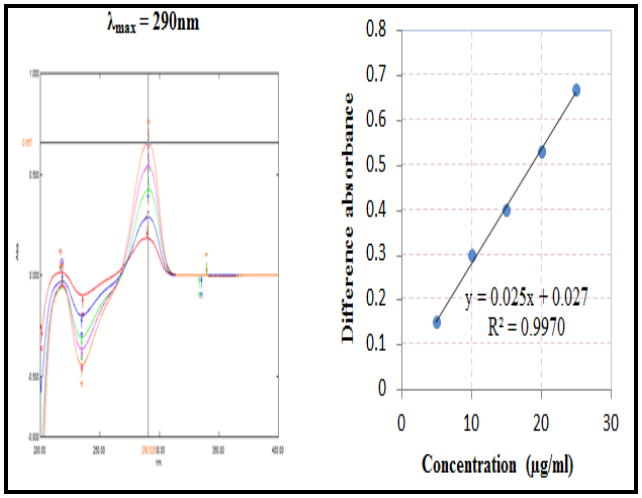

Figure: 1 Overlaid UV- spectra of CDV in the Range of 5-25µg/ml in Water (Reference) and 0.1N HCl (Sample)

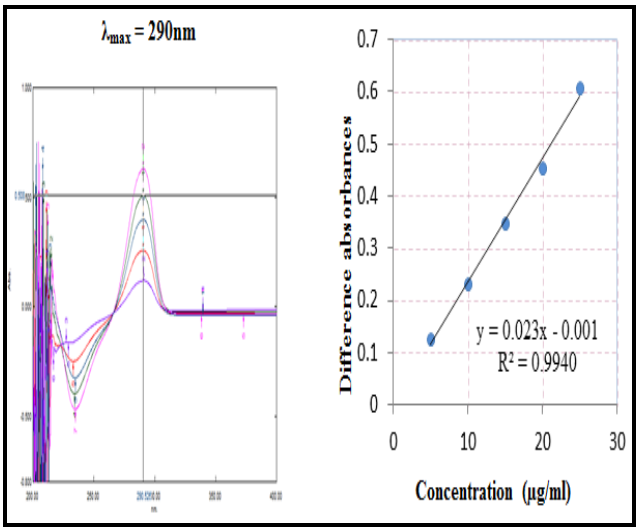

Figure 2: Overlaid UV- spectra of CDV in the Range of 5-25µg/ml in 0.1N NaOH (Reference) and 0.1N HCl (Sample)

Method Validation

Accuracy (% Recovery)

The accuracy study was carried out by spiking the standard solution of CDV to the pre-analysed test solution at three different concentration levels (50, 100,150) and % recovery was calculated. The percentage recoveries of CDV were found in the range of 99.50- 101.72 % and 99.05- 99.90% for above two mention conditions respectively (Table 1).

Precision

The % RSD for repeatability, intra-day and inter-day precision were found to be less than 2% (Table 2, 3 and 4).

Table 2: Results of Repeatability (n=6)

Sample No.	Difference Absorbances Water (Reference) and 0.1N HCl (Sample) (15µg/ml)	Difference Absorbances 0.1N NaOH (Reference) and 0.1N HCl (Sample) (15µg/ml)
1	0.392	0.341
2	0.390	0.338
3	0.392	0.340
4	0.391	0.341
5	0.390	0.341
6	0.391	0.340
Mean	0.391	0.340
SD	0.0008	0.001
%RSD	0.228	0.343

Table 1: Results of Recovery Study (n=3)

Level of Recovery	Amount of Test Solution (µg/ml)	Amount of Standard Solution Added (µg/ml)	Total Concentration (µg/ml)	% Recovery Water (Reference) and 0.1N HCl (Sample)	% Recovery 0.1N NaOH (Reference) and 0.1N HCl (Sample)
0 %	10	0	10	99.50 ± 0.21	99.56 ± 0.43
50 %	10	5	15	101.72 ± 0.14	99.90 ± 0.33
100 %	10	10	20	98.02 ± 0.10	99.05 ± 0.54
150 %	10	15	25	100.83 ± 0.08	99.82 ± 0.34

Table 3: Results of Intra- day and Inter- day precision (n=3) for Water (Reference) and 0.1N HCl (Sample)

CDV (µg/ml)	Intra- Day Precision (n=3)		Inter- Day Precision (n=3)	
	Mean ± SD	%RSD	Mean ± SD	%RSD
5	0.147 ± 0.0011	0.781	0.146 ± 0.001	0.684
s15	0.397 ± 0.0011	0.290	0.395 ± 0.002	0.526
25	0.669 ± 0.0005	0.086	0.668 ± 0.001	0.228

Table 4: Results of Intra- day and Inter- day precision (n=3) for 0.1N NaOH (Reference) and 0.1N HCl (Sample)

CDV (µg/ml)	Intra- Day Precision (n=3)		Inter- Day Precision (n=3)	
	Mean ± SD	%RSD	Mean ± SD	%RSD
5	0.127 ± 0.0008	0.453	0.125 ± 0.0011	0.918
15	0.347 ± 0.0011	0.332	0.345 ± 0.0011	0.334
25	0.606 ± 0.0015	0.251	0.605 ± 0.0005	0.095

LOD and LOQ

The values of LOD for condition-1 and condition-2 were found to be 0.14 and 0.11µg/ml respectively as well as LOQ was 0.48 and 0.39µg/ml respectively.

Robustness

The method was found to be robust as the results were not significantly affected by slight variation in wavelength (±2 nm) (Table 5).

Table 5: Results of Robustness study (Wavelength change by ±2 nm)

Absorption Maxima (nm)	Absorbance Water (Reference) and 0.1N HCl (Sample) (15µg/ml)	% Recovered Water (Reference) and 0.1N HCl (Sample)	Absorbance 0.1N NaOH (Reference) and 0.1N HCl (Sample) (15µg/ml)	% Recovered 0.1N NaOH (Reference) and 0.1N HCl (Sample)
288	0.391	97.06	0.339	98.55
290	0.392	97.33	0.341	98.13
292	0.390	96.80	0.340	98.84
Mean	0.391	97.06	0.340	98.84
SD	0.001	0.266	0.001	0.289
% RSD	0.255	0.274	0.294	0.293

Analysis of Injection Dosage Form

The proposed UV spectrophotometric method was successfully applied for the determination of CDV in injection dosage form. The percentage of CDV using condition-1 and condition-2 were 97.68% and 98.74% respectively (Table 6).

Table 6: Assay of CDV in Injection Dosage Form (n=3)

CDV Injection (75mg/ml)	Amount Recovered for 15µg/ml Solution Water (Reference) and 0.1N HCl (sample)	% Recovery Water (Reference) and 0.1N HCl (sample)	Amount Recovered for 15µg/ml Solution 0.1N NaOH (Reference) and 0.1N HCl (sample)	% Recovery 0.1N NaOH (Reference) and 0.1N HCl (sample)
1	14.60	97.33	14.86	99.13
2	14.72	98.13	14.82	98.84
3	14.64	97.60	14.73	98.26
Mean	-	97.68	-	98.74
SD	-	0.407	-	0.442
%RSD	-	0.416	-	0.447

Table 7: Summary of Validation Parameters

Sr. No.	Parameter	Water (Ref.)- HCl (Sample)	NaOH (Ref.)- HCl (Sample)
1	Linearity (µg/ml)	5- 25	5- 25
2	Regression coefficient (r^2)	0.9970	0.9940
3	Assay (%)	97.68	98.74
4	Accuracy	99.50- 101.72	99.05- 99.99
5	Repeatability (%RSD)	0.228	0.343
6	Intra-day precision (%RSD)	0.08- 0.78	0.25- 0.45
7	Inter-day precision (%RSD)	0.22- 0.68	0.09- 0.91
8	Robustness (%RSD)	0.274	0.293
9	LOD (µg/ml)	0.14	0.11
10	LOQ (µg/ml)	0.48	0.39

CONCLUSION

A Difference spectrophotometric method has been developed and validated for the determination of CDV in injection dosage form. The proposed method was found to be simple, accurate, precise, repeatable and robust. Hence, it can be used successfully for the routine analysis of CDV in bulk and Pharmaceutical formulation in its quality control.

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