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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF CIDOFOVIR DIHYDRATE IN CIDOFOVIR INJECTION

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Cidofovir Dihydrate in Cidofovir Injection form. **Methods:** The drug is soluble in 0.1 N Sodium Hydroxide. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in 0.1 N Sodium Hydroxide. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Cidofovir in injection form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in 0.1 N Sodium Hydroxide, sparingly soluble in Methanol, Ethanol and insoluble in Chloroform. So, the 0.1 N Sodium Hydroxide is used as a diluent in method. The melting point of Cidofovir was found to be 258-259˚C (uncorrected). It showed absorption maxima 290 nm in 0.1 N Sodium Hydroxide. On the basis of absorption spectrum the working concentration was set on 30 µg/ml (PPM). The linearity was observed between 10-50 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 100, 99 and 98.6% for three levels respectively. The % RSD for precision and ruggedness was found to be 0.82% and 0.19% respectively. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Cidofovir in tablet dosage form. The method could be considered for the determination of Cidofovir in quality control laboratories.

KEYWORDS: Cidofovir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Cidofovir (CDF), $C_8H_{14}N_3O_6$ P molecular weight 279.19 g/mol is 1–((3–hydroxy –2– phosphonyl methoxy) propyl) cytosine (Figure 1) with antiviral activity, used against cytomegalovirus 1 (CMV) or retinitis, an infection of the retina of the eye in AIDS patients.[1] CDF in the host cell is changed by reaction specific enzymes into an active product which contains similar structure to one of the pyrimidine base nucleotide of the DNA molecule.[2] Intravenous CDF 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.[3] CDF is a white powder of crystalline nature with a solubility of 170 mg/ml in 0.1 N Sodium Hydroxide at pH 6 to 8. CDF commercially available as an aqueous intravenous injection is 5ml units containing 375 mg of anhydrous CDF and the pH adjusted to 7.4 by a common base or an acid. It was approved by USFDA in May 19962. It is also used for the management of acyclovir- resistant herpes simplex virus infections in immuno compromised patients.[4]

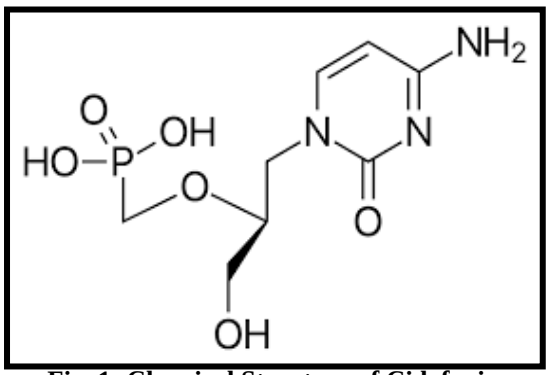

Fig. 1: Chemical Structure of Cidofovir.

From literature review it's found that one method was reported on spectrophotometry for simultaneous estimation, of Cidofovir.[3] Also the method was reported on Related substance determination by HPLC[4], HPLC determination[5,6], as well as HPLC- MS[7] and HILIC[8] for estimation in plasma samples on Cidofovir. But very few methods were reported on estimation of Cidofovir in pharmaceutical formulation form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Cidofovir in injection forms.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

- **Materials**

Cidofovir was obtained as a gift sample. Cidofovir tablets were procured from local pharmacy. 0.1 N Sodium Hydroxide was used throughout the experiment as a diluent. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (30 PPM)[10,11,12]

50 mg weighed amount of Cidofovir was dissolved into 100 ml of volumetric flask with 0.1 N Sodium Hydroxide. Pipette out 3 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with 0.1 N Sodium Hydroxide. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

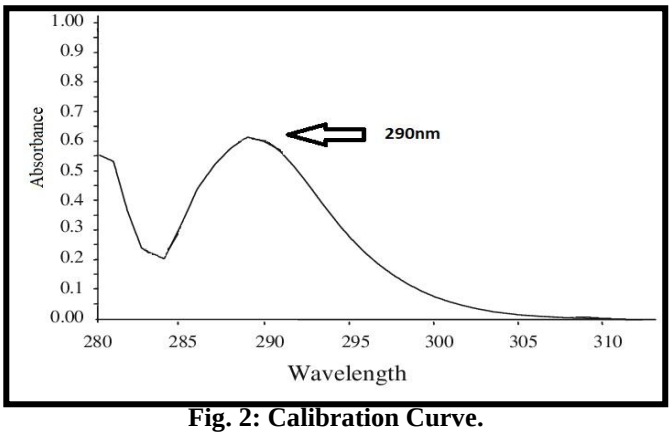

Fig. 2: Calibration Curve.

B. Preparation of Working concentration[10]

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Cidofovir in 100 ml of 0.1 N Sodium Hydroxide to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 3 ml from standard stock solution and diluted up to 50 ml with 0.1 N Sodium Hydroxide to get concentration of 30 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with 0.1 N Sodium Hydroxide. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[10]

For analysis of commercial formulations; Pipette out equivalent to 50 mg of Cidofovir from Cidofovir Injection and transferred into the 100 ml of volumetric flask, added 60 ml 0.1 N Sodium Hydroxide, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with 0.1 N Sodium Hydroxide. Filtered the solution if required through whatmann filter paper. Pipette out 3 ml of the above solution and diluted up to 50 ml with 0.1 N Sodium Hydroxide. The absorbance was measured at 290 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

Emcure Pharmaceutical Limited CIDNVIR™ 500 (375 mg/5mL)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.6467
3	Sample	0.6374

Table 2: Dosage Form Specifications.

Type	Company	M.D.	E.D.	Batch No.	Assay (%)
1	**Emcure Pharma LTD CIDNVIR™ 375 mg/mL**	01/2024	02/2027	23155	98.56

E. Method of validation[9,12-14]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 50 PPM at 290 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	10 PPM	0.2251
2	20 PPM	0.4327
3	30 PPM	0.6341
4	40 PPM	0.8441
5	50 PPM	1.0671
Correlation coefficient		**0.9997**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 50ml of volumetric flask with Analytical Grade 0.1 N Sodium Hydroxide and measuring the absorbance at 290 nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.8	100
100	1	0.99	99.0
120	1.2	1.18	98.55 ~ 98.6

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6442
2	Sample Solution 2	0.6347
3	Sample Solution 3	0.6478
4	Sample Solution 4	0.6384
5	Sample Solution 5	0.6445
MEAN	**0.6419**	
SD	**0.0053**	
% RSD	**0.8204 ~ 0.82**	

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6448	0.6463	99.94	0.1862 ~ 0.19
		0.6478			
2	Analyst 2	0.6421	0.6446	99.68	
		0.6471			

RESULTS

1. Solubility of Cidofovir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	0.1 N Sodium Hydroxide	Freely Soluble
2	Methanol, Ethanol	Practically Insoluble
3	Chloroform	Insoluble

2. Melting point of Cidofovir

The melting point of Cidofovir was found to be 258-259˚C (uncorrected).

3. Results for linearity for assay method of Cidofovir [Conc Vs Absorbance]

The linearity of method was determined at concentration level ranging from 10 to 50 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9997**

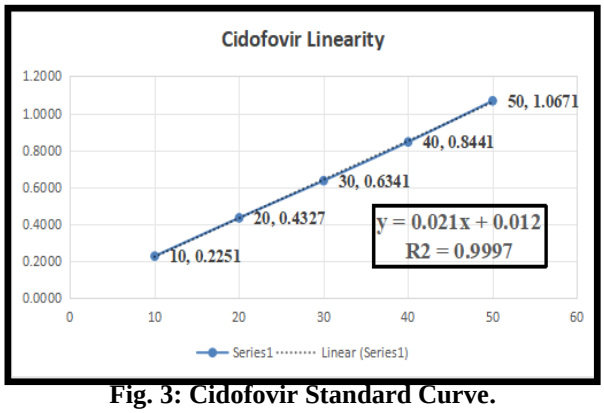

Fig. 3: Cidofovir Standard Curve.

4. Results for accuracy for assay method of Cidofovir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Cidofovir

The % RSD for different sample of precision was found to be 0.82 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Cidofovir

The % RSD for different sample of ruggedness was found to be 0.19 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Cidofovir in tablet form has been developed. From the spectrum of Cidofovir, it was found that the maximum absorbance was 290 nm in 0.1 N Sodium Hydroxide. A good linear relationship was observed in the concentration range of 10-50 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Cidofovir in solid dosage forms. Hence, the method could be considered for the determination of Cidofovir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. DNA - Deoxyribonucleic Acid
4. CMV - Cytomegalovirus 1
5. CDF - Cidofovir
6. LCMS - Liquid Chromatography and Mass Spectroscopy
7. HPLC - High Performance Liquid Chromatography
8. UV - Ultra violet
9. FDA - Food and Drug Administration
10. NaOH - Sodium Hydroxide
11. ICH - International Council for Harmonization
12. RSD - Relative Standard Deviation
13. SD - Standard Deviation
14. Qty - Quantity
15. C - Celsius
16. M.D. - Manufacturing Date
17. E.D. - Expiry Date

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