Development and Validation of a RP-HPLC method for Analysis of Cidofovir in Medicinal Form

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Abstract

Objective: A novel RP-HPLC method has been developed for analysis of cidofovir in its available medicinal form. **Method:** Stationary phase was a sophisticated C-18 RP Column (250mm × 4.6mm). Mobile phase consisted of Ethylnitrile and 0.005M citric acid buffer (pH adjusted to 5.5) in 60:40 V/V ratio. Mobile phase flow rate was 1.0 ml per minute and Isocratic. UV detection wavelength was 260nm. Validation was carried according to ICH guidelines. **Findings:** The Retention time of both AP1 and the medicine was 3.88 minutes. Linearity was satisfied over the concentration range of 2 ppm to 10 ppm and r² value was 0.999. LOD and LOQ values were 0.067 ppm and 0.205 ppm respectively. **Improvement:** The Reported method was novel and all validation parameters met the criteria. Method can be used in quality control analysis.

Keywords: Method Development, Validation, RP - HPLC

1. Introduction

Cidofovir (CDF), $C_8H_{14}N_3O_6P$ molecular weight 279.19 g/mol is 1-((3-hydroxy-2-phosphonyl methoxy) propyl) cytosine (**Figure 1**) with antiviral activity, used against cytomegalovirus (CMV) or retinitis, an infection of the retina of the eye in AIDS patients. 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.

This active product of CDF gets incorporated in to the growing viral DNA strands under the influence of viral DNA polymerase. CDF thus disrupts viral DNA replication in the host cell.

CDF is a white powder of crystalline nature with a solubility of 170 mg/ml in water 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. A medicine that increases uric acid excretion in the urine must be taken before injecting CDF.

Figure 1. CDF Chemical Structure.

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The study of previous works included separation of antiviral compounds², separation of phosphorus containing antiviral compounds³, Analysis of CDF in skin layers⁴, Quantification of CDF in human serum⁵, study of synthesis, transport, and antiviral activity of prod rugs of CDF⁶. The present study focused on a novel RP-HPLC technique development and validation.

2. Materials and Methods

2.1 Chemicals

A sample of CDF (AP1) was obtained from one of the global suppliers of CDF located in west India. Vistide*, CDF injection was purchased from the pharmacy. Ethyl nitrile (EN), Citric acid (CA), Sodium hydroxide (SH) all of analytical grade were purchased from Merck limited. All solutions were prepared using HPLC grade water. All the prepared solutions were filtered and agitated in a ultrasonic probe.

2.2 Instrumentation - (Table 1)

Table 1. HPLC Instrument

Column	C – 18 (250 mm × 4.6 mm)
Decteror	UV – VIS
Pump	Japan made
Injection valve	7725i model, 20 units
Syringe	50 units.
Software	Baseline N2000

2.3 HPLC Conditions (Table 2)

Table 2. HPLC Conditions

Mobile Phase	EN: 0.005M CA 60:40(V/V)
Flow rate (FR)	1ml per minute
Injection volume	20 units
Wavelength	260 nm
Elution type	Isocratic
Buffer pH	5.5

2.4 Preparation of Reagents

2.4.1 CDF Stock Solution

50mg of pure CDF (AP1) was diluted with EN upto the mark in a 50ml volumetric flask. The strength of this solution was 1000 ppm.

2.4.2 CDF Working Standard (WS)

WS of 100 ppm was prepared by diluting 5ml stock solution with EN upto the mark in a 50ml flask.

2.4.3 Sample Solution

100 ppm sample solution prepared by diluting 0.067 ml of VST with EN in a 50ml flask.

2.4.4 Mobile Phase (MP)

MP consisted of a mixture of EN and 0.005M CA buffer in 60:40 V/V ratio.

CA buffer composed of 0.3842 grams of CA in 400 millilitre pure water and its pH adjusted to 5.5 with SH along with stirring.

Based on the solubility studies and after various trials, peak shape, peak tailing factor, theoretical plate count were within the acceptance limit at pH 5.5 for the buffer solution.

2.5 Wavelength Selection

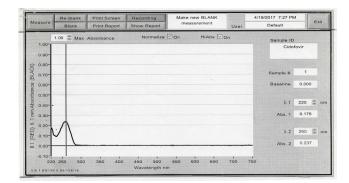


Figure 2. UV - Spectrum.

2.6 System Suitability (SS)

To know the resolution and reproducibility of the method SS study was done.

CDF (WS) was injected to facilitate the study of 6 different chromatograms.

The acceptance criteria for Resolution (Rs), Tailing factor (T), Asymmetry (K), Theoretical plates (N) and percent relative standard deviation (% RSD) for Retention time (RT), Peak area (PA) and Peak height (PH) were checked (Table 3).

Table 3. SS

SS Parameter	Reference Value	Results	
RT	% RSD ≤ 1	0	
PA (n = 6)	% RSD ≤ 1	0.9698	
PH	% RSD ≤ 1	0.7694	
Rs	Rs ≥ 2.0	10.421	
Т	T ≤ 2.0	0.944	
K	K ≤ 2.0	0.958	
N	N ≥ 2000	6694.2	

2.7 Estimation of CDF in Sample

The Response factor (RF) of the WS and the sample were calculated separately from the average of six peaks to calculate the amount of the drug.

Amount of the drug = $\frac{RF \text{ of the sample}}{RF \text{ of the } WS} \times \text{Strength of}$ the WS

2.8 Method Validation

Validation done as per ICH Q2 (R1) guidelines² for linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD) and limit of Quantification (LOQ).

3. Results and Discussion

3.1 SS

All the SS measurements met the criteria (Table 3).

3.2

RT of the Blank (Mobile phase) was 2.008 minutes (Figure 3).

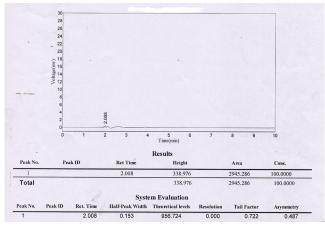


Figure 3. Blank Chromatogram.

RT of the WS was 3.88 minutes (Figure 4).

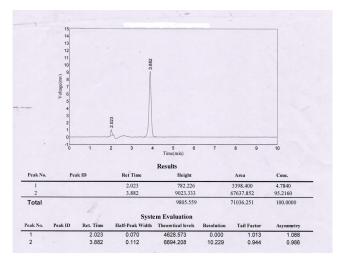


Figure 4. CDF WS.

RT of the sample was 3.88 minutes (**Figure 5**).

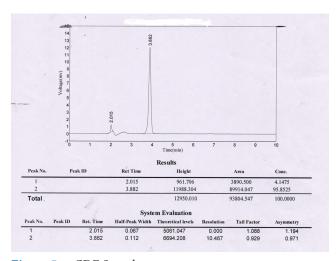


Figure 5. CDF Sample.

3.3

The amount of drug in VST was found to be 99.97% approximating the label claim.

3.4 Linearity

Five solutions levels ranging from 2ppm to 10 ppm were prepared from the WS. Each level in the range was injected thrice.

The calibration curve of PA versus strength in ppm (microgram per millilitre) was plotted.

The regression equation and the correlation coefficient (r^2) value depicted linearity between PA and the concentration of the sample solution (Figure 6) (Table - 4A and 4B).

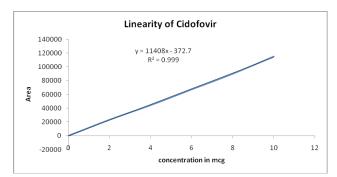


Figure 6. Linearity.

Table 4A. Linearity

Strength (ppm)	PA (n = 3)	% RSD
2	23300.53	0.78
4	44329.4	0.91
6	67276.916	0.46
8	89964.76	0.42
10	115117.169	0.63

Table 4B. Linearity

<u> </u>				
Regression Equation $y = 11408x - 372.77$				
Slope (m) = 11408				
Intercept (C) = -372.7				
S D of (C) = 233.9				
$R^2 = 0.999$				

3.5 Accuracy

80%, 100% and 120% of WS solutions were spiked to sample solutions of 20 ppm and each injected thrice. The average PA study depicted a percent recovery of 101.19, 100.41 and 99.22 from each of the WS. and met the criteria limits.

% RSD for PA was less than 1 (**Table 5**).

Table 5. Accuracy

Sample	% of WS	Amount WS (ppm)		PA (n = 3)	% RSD	Recovery (%)
		Spike Found				
1	80	4.8	4.85	55813.9	0.5845	101.04
2	100	6.0	6.02	68943.1	0.8928	100.33
3	120	7.2	7.14	82093.1	0.9151	99.16

3.6 Precision

Studied with 6 ppm sample solution. Six separate chromatograms taken on the same day (Intraday precision) (**Table 6**) and an average of three chromatograms taken separately on three different days (Interday precision) (**Table 7**) were studied to know the % RSD of RT and PA. All the values met the criteria and confirm the closeness of the data to each other and hence the method was precise.

Table 6. Intraday Precision

Trial	PA	RT	PH	
1	69747.953	3.88	9248.421	
2	69498.953	3.88	9153.844	
3	70650.148	3.88	9306.481	
4	69911	3.88	9240.222	
5	70769.602	3.88	9309.593	
6	69004.102	3.88	9146.806	
Mean	69930.293	3.88	9234.227	
SD	678.25	0	71.052	
% RSD	% RSD 0.9698 0		0.7694	

Table 7. Interday Precision

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Trial	Day 1 (PA)	Day 2 (PA)	Day 3 (PA)			
1	1 70120.5 70103.89		69134.1			
2	69778	69639.89	70365.89			
3	70705.89	70935	69610.29			
Mean	70201.48	70226.26	69703.44			
Std.Dev	469.2	656.16	621.13			
% RSD	0.668	0.934	0.891			

3.7 LOD - 0.067 ppm

3.8 LOQ - 0.205 ppm

3.9 Robustness

Small variation in the experimental conditions (**Table 8**). Showed little or no affect on the average PA, RT, and % RSD values and hence the method was Robust (**Table 9**).

 Table 8.
 Experimental Conditions

Value	I	II	III
Buffer pH	5.4	5.5	5.6
Column Temp (°C)	34	35	36
MP (FR) (ml/min)	0.98	1.00	1.02

Table 9. ROBUSTNESS

Value	рН			Temp		FR			
	5.4	5.5	5.6	34	35	36	0.98	1.00	1.02
Mean PA (n = 3)	70449.9	69892	70381	71638	71807	71970	73197	70739	70697
SD	313.44	682.97	371.05	288.6	608.6	707.9	172.9	109.7	674.1
% RSD	0.4449	0.9771	0.5272	0.40	0.84	0.98	0.236	0.155	0.953

4. Conclusion

The developed RP-HPLC method was simple, rapid accurate and selective. All the validation measurable for the AP1 and the sample were similar. The method can be used in quality control test.

5. References

- 1. Cidofovir Monograph. Available from: Crossref. Date accessed: 13/07/2017.
- 2. Loregian A. Separation methods for Acyclovir and related antiviral compounds. Journal of Chromatography B. 2001; 764(1-2):289-311. Crossref.
- 3. France B. Separation methods for Antiviral phosphorus containing drugs. Journal Of Chromatography B. 2001; 764(1-2):1801-11.

- 4. Santoyo S, de Jalon EG, Campanero MA, Ygartua P. Determination of Cidofovir in both skin layers and percutaneous penetration samples by HPLC. Journal of Pharmaceutical and Biomedical Analysis. 2002;29(5):819-26.
- 5. Breddeman A, Hsien L, Tot E. Quantification of Cidofovir in human serum by LC-MS/MS for children. Journal of Chromatography B. 2008; 861(1):1-9. Crossref. PMid:18083075.
- 6. Eriksson U, Peterson W, Boris A. Serine Peptide Phosphoester prodrugs of Cyclic Cidofovir. Synthesis, Transport and Antiviral activity. Molecular Pharmaceutics. 5(4):598-609. Crossref. PMid:18481868 PMCid:PMC2629803.
- 7. Q2 (R1) Validation Of Analytical Procedures: Text and Methodology.