

HPLC METHOD DEVELOPMENT OF CIDOFOVIR AS BULK DRUG AND ITS FORMULATION

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Abstract: A simple HPLC method was developed and validated for quantitation of cidofovir in pure form. The HPLC separation was achieved on a C₁₈ 5 μm Waters column (250 mm × 4.6 mm) using a mobile phase of methanol – water (20:80, v/v) containing 10% NaOH to adjust pH6.2 at a flow rate of 1.0 ml/min. The UV detector was operated at 270 nm. The method was validated for specificity, linearity, precision, robustness and limit of quantitation. The degree of linearity of the calibration curves, limit of detection and quantitation for the HPLC method were determined. The method was found to be simple, specific, precise, accurate, and reproducible

Keywords- Cidofovir, Methanol, Water, RP-High performance liquid chromatography.

I. INTRODUCTION

(([(S)-1-(4-AMINO-2-OXO-1,2-DIHYDROPYRIMIDIN-1-YL)-3-HYDROXYPROPAN-2-YL]OXY)METHYL) PHOSPHORIC ACID HAVING MOLECULAR FORMULA C₈H₁₄N₃O₆P AND MOLECULAR MASS IS ABOUT 279.187 G/MOL. MOLECULE IS POLAR AND ACIDIC IN NATURE, AND CAN ALSO FORM A CYCLIC BY-PRODUCT. CIDOFOVIR AND CYCLO-CIDOFOVIR WERE SEPARATED ON A COLUMN BASED ON THEIR WEAK HYDROPHOBIC AND STRONG ACIDIC PROPERTIES. CIDOFOVIR IS IN WHITE POWDER FORM. SELECTIVITY OF SEPARATION CAN BE CONTROLLED BY THE AMOUNT OF WATER AND AMOUNT OF METHANOL IN THE MOBILE PHASE. ELUTION CAN BE MONITORED BY UV, LC/MS. (FIG. 1).

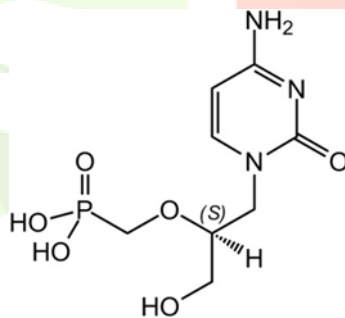


Fig. 1 Chemical Structure of Cidofovir

The structural formula of Cidofovir is: The literature reveals that numerous analytical methods have been reported for the determination of cidofovir in pharmaceutical preparations and human serum. These methods are based on Gas chromatography (GC) . Gas chromatography - Mass spectrophotometry (GC-MS) , HPLC and fluorometry. The GC methods require complex sample preparation involving double derivatization of the drugs to improve the volatility and avoid column interactions. Fluorometric methods are less accurate and less specific than HPLC. This paper describes a sensitive, fast, simple and economical method for the determination of cidofovir in pure form.

II. EXPERIMENTAL:

2.1 Reagents and chemicals:

Cidofovir used as an internal standard. (M. Cure Pharma Ltd. Pune)

HPLC grade Methanol. (Research lab fine chem. Industries-Mumbai)

HPLC grade Water. (Merck specialities private limited- Worli, Mumbai)

All other chemicals were of analytical grade and used without any further purification.

2.2 Apparatus:

Volumetric Flask, Beaker, Pipette, Funnel.

2.3 Instrument:

- The HPLC used was model PU-2080, Jasco, Tokyo, Japan with pump model PU-2080 Intelligent HPLC Pump.
- The detector was a UV detector model UV-2075, Japan.
- EQUIP- TRONICS Digital pH- meter, Japan.
- SHIMADZU 1800 UV spectrophotometer, Japan.
- Infra-Red spectroscopy.
- SHIMADZU Electronic balance, Japan.

2.4 Chromatographic System And Conditions

HPLC method was performed using a PU- 2080 Intelligent Pump. The mobile phase consisted of methanol – water (20:80, v/v) at a flow rate of 1.0 ml/min. Final pH of the mobile phase was adjusted to 6.2 by 10 % NaOH

2.5 Preparation of Mobile Phase

Weigh accurately about 200ml HPLC grade water and mix with 800ml HPLC grade methanol and mixed well. The resulting solution was sonicated for 5min using ultrasonic bath, and finally this solution was filtered using 0.2µm filter.

2.6 Stock solution preparation:

The stock solution of cidofovir was prepared by dissolving 100mg of standard cidofovir to 100ml with HPLC grade methanol to give a concentration of 1000 µg/ml. The solution was sonicated for 5min using ultrasonic bath and then filtered through 0.2µm disk filter.

2.7 Sample Preparation:

Aliquots of stock solution (10 mg/ml) were pipette into a series of 10 ml volumetric. To each flask, 1.0 ml were added and diluted to volume with distilled water. The calibration curve was constructed by plotting peak area against the initial concentration of cidofovir. The linearity range or Beer's range follows in the range between 30 to 120 µg/ml. The content of cidofovir was calculated either from the calibration curve or corresponding regression equation. The isocratic mobile phase pumped at a flow rate of 1.0 ml/min consisted of methanol - water.

III. ANALYTICAL METHOD VALIDATION PARAMETERS

3.1 System Suitability

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

3.2 Linearity

The linearity was analyzed through the standard curves ranging from 30 to 120 µg/ml by diluting appropriate amounts of cidofovir stock solution (1000 µg/ml) with HPLC grade methanol and prepared in triplicate. Three calibration curves were prepared in the same day with the following concentrations (30, 60, 90, 120, and 150µg/ml). The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

3.3 Specificity

The specificity of the developed HPLC method for the determination of cidofovir in bulk drug.

3.4 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 (100µg/ml). The results were expressed as %RSD of the measurements.

3.5 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.

3.6 Ruggedness

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

3.7 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.

IV. RESULTS AND DISCUSSION:

A. Method Validation

1. System Suitability

Parameter	Value (Mean \pm %RSD)*
Peak area	3457550 \pm 0.096
Tailing factor	1.109 \pm 0.378
Theoretical plate	2945.667 \pm 0.099
Retention time	4.581 \pm 0.083

Table 1: Chromatographic characteristics of system suitability solution

2. Linearity

The regression equation for cidofovir was found $y=17563x-50470$ by plotting peak area (y) versus the concentration (x) studied from 30 to 120 $\mu\text{g/ml}$, and the correlation coefficient ($R^2=0.999$) 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 ($P<0.05$).

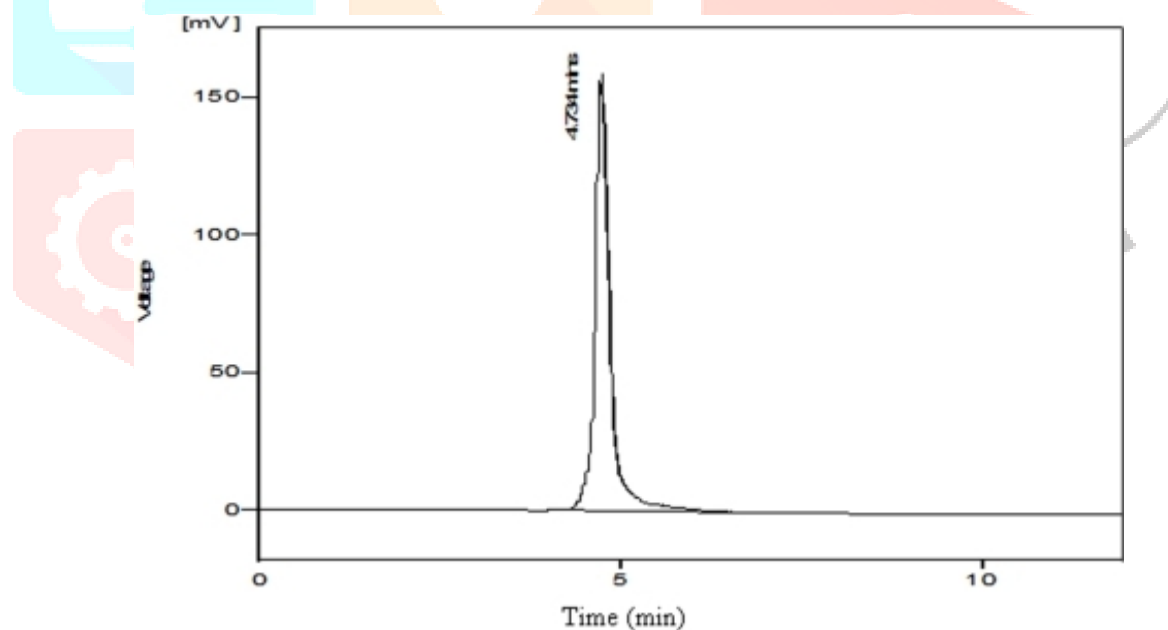


Table 3: Specificity of Cidofovir

3. Specificity

A typical HPLC chromatogram of cidofovir standard preparation is shown in Figure. The HPLC chromatograms recorded for the standard drug of peak purity was 99.99%.

4. 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.

Sr. No	Concentration ($\mu\text{g/ml}$)	Intra-day precision	Inter-day precision
1	100	3479501	3484566
2	100	3479005	3484112
3	100	3478987	3483991
4	100	3479227	3484977
5	100	3479808	3485004
6	100	3479777	3484669
Mean	-	3479384	3484553
S.D	-	367.100	425.941
% R.S.D	-	0.011	0.012

Table 4: Intraday and Interday precision of HPLC method

5. Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations $\text{LOD} = 3 \times \sigma / S$ and $\text{LOQ} = 10 \times \sigma / S$, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be $0.0968 \mu\text{g/ml}$ and respectively for zero order derivative and The LOD and LOQ were found. $0.2904 \mu\text{g/ml}$

6. Ruggedness

The results (% of Recovery \pm Standard Deviation of six assay samples) are given in Table, indicating the ruggedness of the current method.

Parameter	Taken amount of standard cidofovir (mg)	Analyst-1		Analyst-2	
		Amount found (mg)	% Recovery \pm SD*	Amount found (mg)	% Recovery \pm SD*
Sample I	10	10.04	100.4 \pm 0.08	10.01	100.1 \pm 0.09
Sample I	10	10.03	100.3 \pm 0.06	10.03	100.3 \pm 0.05
Sample I	10	10.01	100.1 \pm 0.04	10.07	100.7 \pm 0.02

Table 5: Ruggedness of HPLC method

7. Robustness

The % of RSD of robustness testing under different altered conditions is given in Table 6, indicating that the current method is robust.

Parameter	Amount of cidofovir added ($\mu\text{g/ml}$)	Amount of cidofovir detected (Mean \pm SD)*	%RSD
Change in mobile phase composition	100	100.33 \pm 0.29	0.14
	100	100.47 \pm 0.29	0.14
	100	100.56 \pm 0.55	0.27
Change in column temperature	100	100.43 \pm 0.71	0.35
	100	100.36 \pm 0.50	0.23
	100	99.88 \pm 0.56	0.28
Change in flow rate	100	100.08 \pm 0.65	0.33
	100	100.41 \pm 0.31	0.15
	100	100.46 \pm 0.61	0.31

Table 6 : Robustness of HPLC method.

V. CONCLUSIONS

The developed RP-HPLC method for the determination of cidofovir is simple, precise, accurate, reproducible, and highly sensitive. The developed method was validated based on USP and ICH guidelines. Hence, this method can be used for the routine determination of cidofovir in pure form. This method is mainly economically because in this method stock solution is prepared by methanol but further dilutions Prepared are by Distilled Water.

VI. ACKNOWLEDGMENT

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