Journal of Innovations in Applied Pharmaceutical Science [JIAPS]

Content available at: www.saap.org.in ISSN: 2455-5177

A BRAND-NEW EFFICIENT AND PRECISE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF CIDOFOVIR AND FAMCICLOVIR

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Article History

Received: 21-08-2023
Revised: 11-09-2023
Accepted: 04-10-2023

Keywords:
Simultaneous determination, Validation, ICH Guide lines, RP-HPLC, Cidofovir, Famciclovir.

Abstract

The quantitative analysis of cidofovir and famciclovir using a Symmetry C-18 150x4.6mm, 3.5 column with a flow rate of 1ml/min has resulted in the development of a brand-new, effective, and exact high performance liquid chromatographic method. Acetonitrile and buffer are mixed in 60:40 ratios for the mobile stage, and the buffer is created by dissolving 1 mL of formic acid in 1 liter of HPLC water. The detection was done at a wavelength of 250 nm. After 8 minutes of running time, the Cidofovir and Famciclovir peaks were separated, with the former peak eluting after 2.8 minutes and the latter peak eluting after 6.4 minutes, respectively.The proposed method displays strong linearity in the concentration ranges of 7.5 g/ml to 112.5 g/ml for cidofovir and 25 g/ml to 375 g/ml for famciclovir. The findings of the precision and recovery examinations range from 98 to 102%. The percent RSD is less than 2.0% in any robustness scenario. Under pressure, deterioration has little impact, and solutions remain effective for the entire day. The parameters of precision, accuracy, specificity, stability, robustness, linearity, limit of detection, and limit of quantification were evaluated and found to be within the acceptable range when the method was devised and validated in accordance with ICH guidelines.

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https://doi.org/10.37022/jiaps.v8i3.504

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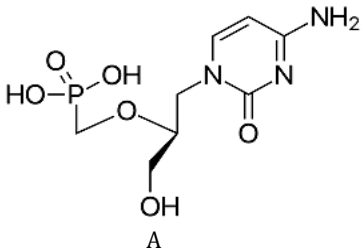
Introduction

The injectable antiviral drug cidofovir, which is marketed under the brand name Vistide, is principally used to treat cytomegalovirus (CMV) retinitis [1] in AIDS patients. Cytomegalovirus retinitis is the only symptom recognized by regulatory bodies worldwide, and cidofovir is effective in treating HSV infections that are resistant to aciclovir. Furthermore, intriguing research on cidofovir as a potential treatment for progressive multifocal leukoencephalopathy has been conducted [3]. Randomized tests, however, revealed that the medication was useless [4] In the event of a bioterror attack involving smallpox cases; cidofovir might have smallpox prevention properties and could be used in a limited capacity. Brincidofovir is an oral cidofovir substitute with significantly increased smallpox effectiveness [5]. Despite the lack of clinical studies to date, it has inhibitory effects on the replication of the varicella-zoster virus in vitro [6], likely as a result of the accessibility of safer alternatives such aciclovir [7]. In a limited number of transplant recipients, cidofovir shows anti-BK virus activity [8].

Herpes zoster and other herpesvirus infections are treated with the antiviral drug famciclovir, which contains guanosine analogues [9] (shingles). It is a penciclovirprodrug with a higher oral bioavailability. The antiviral drug famciclovir, also known by the brand name Famvir (Novartis), is used to treat immune-competent patients' herpes labialis (cold sores), herpes zoster (shingles), and herpes simplex virus 2 (genital herpes), as well as to prevent recurrent outbreaks (Tyring S et al., 1995). Additionally, HIV patients who have persistent herpes simplex are treated with it.The chemical structures of Cidofovir and Famciclovir are shown in Figure 1.



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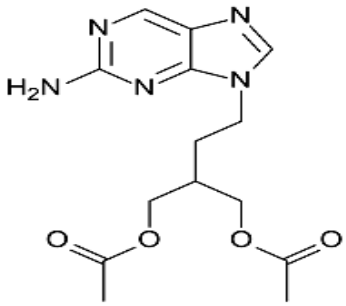

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Fig. 1: Chemical structure of (A) Cidofovir and (B) Famciclovir

Materials and Methods

Materials: Acetonitrile, formic acid and water were purchased from Merck (India) Ltd. Worli, Mumbai, India. Glenmark Pharmaceuticals, Mumbai supplied the APIs for Cidofovir and Famciclovir, which were used as benchmarks.

Equipment: HPLC creates: The Waters alliance e-2695 chromatographic device was used, which included quaternary pump, PDA detector-2996 and chromatographic programme Empower-2.0.

Chromatographic Conditions: The method was created and verified using an HPLC system instrument (Waters Alliance e2695 model). Data processing was carried out using Empower 2.0 software. The column's measurements were Symmetry C-18 150x4.6mm, 3.5. Isocratic elution was used to separate the chosen molecule, and the mobile phase consisted of acetonitrile and 0.1 % formic acid buffer solution in 60:40 ratio. The pump flow rate was set to 1.0 ml/min. The wavelength used for UV detection was 250 nm. The mobile process's diluent was employed as the injection fluid, and the injection volume was set at 10 liters.

Preparation of 0.1 % formic acid: 1ml formic acid was dissolved in 1 liter of HPLC grade water and filtered through 0.45µ filter paper.

Mobile Phase Preparation: Acetonitrile 0.1 %t formic acid (60:40).

Diluent: Mobile Phase was used as diluent.

Standard solution Preparation: Weigh 75 mg of Cidofovir and 250 mg of Famciclovir working requirements into a 100ml volumetric flask, add 70ml of diluents, sonicate for 15 minutes to remove the contents, and dilute volume with diluent. Dilute 1 mL to 10 mL with diluents.

Optimization of Wavelength: Using a PDA detector, the absorption spectra of Cidofovir and Famciclovir solutions were scanned and registered over the 200-400 nm range. Looking at the spectrum, we can see that Cidofovir and Famciclovir have the highest absorbance at 250 nm. As a result, the method validation wavelength of 250 nm was chosen.

Results and Discussion

Optimization of Method

The reversed-phase HPLC system uses Symmetry C-18 150x4.6mm, 3.5 columns with isocratic elution as the initial chromatographic state. The mobile method makes use of a mixture of buffer and acetonitrile (60:40). The column temperature is ambient, and the flow rate is 1.0 ml/min. The finalized diluent and standard solution concentrations, as well as injection volumes greater than the quantification maximum, are used to determine the recovery data and peak sharpness (LOQ). To reach the optimal resolution, the isocratic process was adjusted. The parameters of the developed and validated HPLC procedure with the optimized chromatographic conditions are shown in Table 1.

S. No.	Parameter	Method Conditions
1	Column	Symmetry C-18 150x4.6mm, 3.5µ
2	Flow rate	1 ml/min
3	Wave length	250nm
4	Injection Volume	10µl
5	Run time	8 min
6	Mobile phase	0.1% formic acid: ACN 60:40

Table 1: Optimized HPLC method conditions.

System suitability: The HPLC system's system suitability parameters were discovered to be within acceptable bounds after the addition of the standard solution. Standard peak areas were used to calculate the percentage of RSD. The acceptable range was found for the RSD percentage of identical injections. Table 2 displays the results obtained, while Figure 2 displays the device suitability chromatogram.

S. No	System suitability parameter	Acceptance criteria	Drug Name	
			Cidofovir	Famciclovir
1	% RSD	NMT 2.0	0.55	0.14
2	USP Tailing	NMT 2.0	1.01	1.05
3	USP Plate count	NLT 3000	2459	7365

Table 2. Results of system precision.

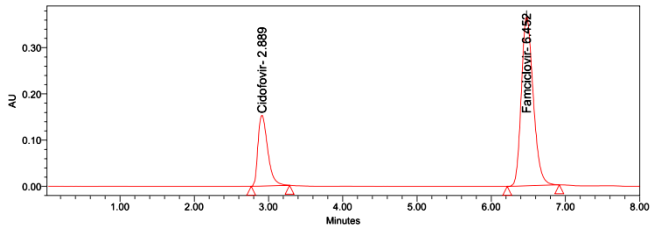


Fig. 2: Chromatogram of standard.

Specificity: A study was carried out to assess the placebo effect. The HPLC equipment is filled with standard solutions containing equal weights of API and placebo in accordance with the test method. At the retention times of cidofovir and famciclovir, there was no interference in the chromatograms of placebo solution or empty cell solution. The chromatograms of the placebo and blank solutions showed no interference at the Cidofovir and Famciclovir retention times. Figure 3 shows a chromatogram that is empty.

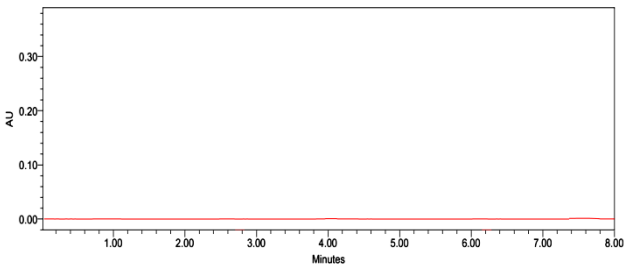
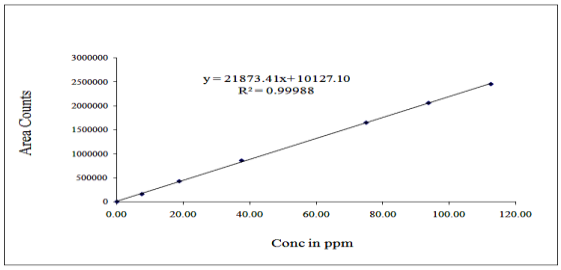


Fig. No. 3: Chromatogram of blank

Linearity: Linearity concentrations ofCidofovir and Famciclovir were prepared in the ranges of 7.5 g/ml to 112.5 g/ml and 25 g/ml to 375 g/ml respectively. The regression equation for cidofovir was Y=21873.41x+10127.10 (CC-0.9998), while Famciclovir's was Y=14129.08x+5862.72 (CC-0.9998) (CC-0.9997).Figure 4 depicted the linearity map, and table 3 displayed the effects.



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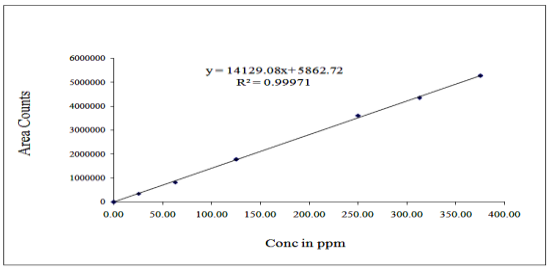

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Fig. 4: Linearity plot of (A) Cidofovir and (B) Famciclovir

S. No.	Cidofovir		Famciclovir	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	7.50	158204	348542	348542
Linearity-2	18.75	425693	823021	823021
Linearity-3	37.50	859647	1785496	1785496
Linearity-4	75.00	1652476	3618462	3618462
Linearity-5	93.75	2063021	4353621	4353621
Linearity-6	112.50	2458174	5278263	5278263
Slope	21873.41		14129.08	
Intercept	10127.10		5862.72	
CC	0.99988		0.99971	

Table 3: Results of Linearity

Robustness: There is no appreciable change in RSD in Robustness despite a negligible difference in flow rate (0.2ml) and organic solvent (10%) in their chromatographic state. The outcomes are shown in Table 4.

S.No	Parameter name	% RSD for purity	
		Cidofovir	Famciclovir
1	Flow (0.8ml/min)	1.45	0.94
2	Flow (1.2ml/min)	1.01	0.23
3	Organic solvent (+10%) (44:56)	0.69	1.64
4	Organic solvent (-10%) (36:64)	0.88	0.25

Table 4: Results of Robustness

Precision: By injecting test preparation and testing the complete analytical process from standard solution preparation to the end result, the precision of the method was determined. A minimum of six determinations were utilized to determine repeatability, and the percent relative standard deviation was computed. Table 5 provides a summary of the results.

Analyte	Standard Conc.	%RSD
Cidofovir	75	0.39
Famciclovir	250	0.47

Table 5: Results of Method precision

Intermediate Precision: Six samples of a standard solution were examined using a separate analyst and instrument on a different day. Peak areas, mean values, and % RSD values were determined. Table 6 summaries the results obtained.

Analyte	Standard Conc.	%RSD
Cidofovir	75	0.58
Famciclovir	250	1.15

Table 6: Results of Intermediate precision

Limit of Detection and Quantification (LOD &LOQ): The calibration curve approach was used to calculate LOD and LOQ. An established RP-HPLC procedure was used to determine the compound's LOD and LOQ by injecting standard solutions at progressively decreasing concentrations. Equations LOQ= 10x/S and LOD= 3.3x/S, respectively, were used to estimate LOD and LOQ using the slope method, where S is the calibration curve slope and is the response standard deviation. Cidofovir's LOD and LOQ values were 0.094 g/ml and 0.310 g/ml, compared to Famciclovir's 0.313 g/ml and 1.033 g/ml.

Accuracy: Recovery studies at three different concentration levels 50%, 100%, and 150% were carried out to ascertain accuracy. Cidofovir concentrations of 37.5, 75, and 112.5 g/ml and Famciclovir concentrations of 125, 250, and 375 g/ml were used to make APIs. It was found that the recovery rates ranged from 98 to 102%. The accuracy figures are shown in Tables 7 and 8.

S. No.	% Level	% Recovery	Ave % Recovery
1	50	100.2	100.2
2		100.4	
3		100.1	
4	100	99.6	98.8
5		98.1	
6		98.6	
7	150	99.5	99.7
8		99.8	
9		99.8	

Table 7: Results of Accuracy of Cidofovir

S. No.	% Level	% Recovery	Ave %Recovery
1	50	98.6	99.2
2		99.7	
3		99.2	
4	100	100.2	99.4
5		99.7	
6		98.4	
7	150	100.4	100.4
8		100.7	
9		100.2	

Table 8: Results of Accuracy of Famciclovir

Degradation Effects: The Cidofovir and Famciclovir standard stocks were subjected to various forced degradation conditions in order to influence partial drug degradation. Experiments with forced degradation were carried out to demonstrate that the process works with deteriorated goods. Furthermore, the experiments reveal the conditions under which the medication becomes unstable, allowing precautions to be taken during formulation to avoid potential instabilities.

Acid Degradation: The acid degradation procedure involves adding 1ml of 1N HCl, 1ml of 1N NaOH, and 5ml of standard solution in to a 50ml volumetric flask. The flask is then heated for 30 minutes at 60°C. A 0.45 nylon syringe filter is then used to filter the fluid.

Alkali Degradation: In the alkali degradation procedure, 1ml of 1N NaOH is poured to a 50ml volumetric flask along with 5ml of the standard solution. The flask is then heated for 30 minutes at 60°C before 1ml of 1N HCl is added and the flask is marked with diluent. A 0.45 nylon syringe filter is then used to filter the fluid.

Peroxide Degradation: The process of breakdown was as follows: A 50 mL volumetric flask was filled with 5 mL of the standard solution and 1 mL of the 30% $H_2O_2$and heated for 30 minutes at 60 °C before cooling to make the diluent. A 0.45 nylon syringe filter is used to filter thesolution.

Reduction Degradation: The degradation procedure was as follows: In a 50 mL volumetric flask, 5mL of regular solution were mixed with 1mL of a 30 %$NaHCO_3$ solution. In order to prepare the diluent, the mixture is heated at 60^0C for 15 minutes. A 0.45 nylon syringe filter is used to filter the fluid.

Thermal Degradation: The exposed standard solution was examined following a three-hour exposure at 105°C of 500 mg and 1000 mg of cidofovir and famciclovir, respectively. 10ml of the volumetric flask were filled with 5mg of the standard solution. A sonicater was used to evenly distribute 5 mL of the diluent before it was diluted to volume. This mixture is put into an RB flask and refluxed for 60 minutes at 60 °C. Allow it to cool to room temperature after that. Utilize diluents to diluted 1ml to 10ml.

UV Degradation: The regular solution was refluxed at 60°C for 30 minutes for the UV degradation procedure after being out in the sun for 12 hours. The HPLC method was used to inject the regular solution.

Hydrolysis Degradation: The hydrolysis degradation phase is performed by transferring 5 ml of the standard solution into a 50 ml volumetric flask, adding 2 ml of HPLC water, heating for 15 minutes at 60 °C, and cooling to make up with diluent. Apply a 0.45 nylon syringe filter to the fluid to filter it. The results of forced deterioration are shown in Table 9.

Degradation Condition	% Degradation of Cidofovir	% Degradation of Famciclovir
Unstressed Degradation	99.8	99.9
Acid Degradation	14.6	13.4
Alkali Degradation	14.9	14.2
Peroxide Degradation	15.2	14.7
Reduction Degradation	13.9	12.8
Thermal Degradation	12.5	11.4
Photolytic Degradation	10.7	12.9

Table 9: Forced degradation results

Conclusion

A stable indication test for cidofovir and famciclovir was developed using a validated RP-HPLC method. The drug's degradation behavior was examined in a variety of conditions, including (acid, base, and neutral), oxidation, reduction, photolysis, and heat stress. The drug was shown to be unstable in the other degradation scenarios but stable in thermally neutral conditions. It has been possible to detect cidofovir and famciclovir using an efficient isocratic RP-HPLC method. The regression line equation can accurately predict the drug concentration in the range of 7.5-112.5 g/ml for cidofovir and 25-375 g/ml for famciclovir based on the measured peak area. The technique was effectively tested and provided for the precise, quick, reliable, and fast detection of cidofovir and famciclovir.

Funding

The authors declare that they have no funding support for this study.

Acknowledgement

I thankful to Management PACE Institute of technology and sciences for encouragement and supporting to finish this research work.

Conflict of Interest

The authors declare that there is no conflict of interest for this study

Author Contribution

All authors have contributed equally.

Informed Consent

This manuscript not published at any other journals.

Ethical Statement

This study does not involve experiments on animals or human subjects.

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