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Validated RP-HPLC Method for the Quantitation of Ganciclovir In Bulk and Capsule Dosage Form

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ABSTRACT

A simple, specific, accurate, precise and sensitive RP- HPLC method has been developed for the rapid estimation of Ganciclovir in bulk and its formulations. The chromatographic separation was carried on Grace smart RP-18 column (250 x 4.6 mm, 5 µm), using Methanol: Citrate buffer (0.05M) at PH-5.2 with KOH 70:30 (v/v) as mobile phase, at a flow rate of 1.0 ml/min. The detection was carried out at 254 nm and drug eluted with a retention time of 2.982 min. Beer's law was obeyed in the concentration range of 10-60µg/ml with correlation coefficient 0.999. The method has been validated according to ICH guidelines for specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. The method was found to be specific, accurate, and precise, robust, rugged and sensitive. The developed method was good linearity, novel, rapid for the estimation of Ganciclovir in bulk and capsule dosage form. Thus it can be employed for the routine analysis.

Keywords: Ganciclovir , RP-HPLC, Validation, Citrate buffer.

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INTRODUCTION

Ganciclovir (GCV) [1, 2] is chemically 2-amino -1,9-[{2-hydroxy -1-(hydroxymethyl) ethoxy} methyl]-6-H-purine -6-H-one, is a synthetic nucleoside analogue closely related to Acyclovir. It is used in the treatment of cytomegalovirus (CMV) infection in AIDS patients. GCV exhibits antiviral activity against herpes simplex virus (HSV) and cytomegalovirus (CMV) at relatively low inhibitory concentrations. Ganciclovir is a white crystalline powder and soluble in Hydrochloric acid, water, methanol, ethanol, and dimethyl sulphoxide. Its molecular weight 255.23g/mol.

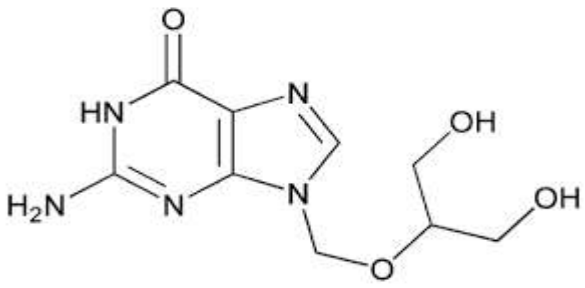

Figure 1: Chemical Structure of Ganciclovir.

Literature survey reveals that UV spectrometric[3-8], and RP-HPLC[9-12] method reported for Ganciclovir. The aim of present work was to develop and validate a novel, rapid, simple, precise, sensitive and specific RP-HPLC method for estimation of Ganciclovir in its bulk and capsule dosage form.

MATERIALS AND METHOD

Instrument:

Chromatographic separation was performed on a Shimadzu LC-10ATVP HPLC system comprising a Shimadzu spd10A uv-vis detector, Shimadzu LC-10ATVP pump and enable Grace smart RP18 column (250 x 4.6 mm i d, 5µm particle). A manually operating Rheodyne injector 50µl (20µl injection valve) was used for injecting sample and standard solution. Baseline chromatography Data system N2000 software was used to collect and process the data.

Chemicals and Reagents:

Ganciclovir pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form Natclovir 250 capsules labelled claim 250 mg were purchased from local pharmacy manufactured by NATCO LTD. Methanol of HPLC grade(Merck India), Water of HPLC grade(Merck India) and citrate of Analytical grade (SD Fine Chemicals) were used.

Selection of mobile phase:

Based on sample solubility, stability and suitability various mobile phase compositions were tried

to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, Methanol: Citrate buffer (0.05M) at PH-5.2 with potassium hydroxide (70:30) was chosen with detection wavelength 254 nm, since it gave sharp peak with good symmetry within limits.

Buffer preparation: Citrate (0.05M)

Citric acid solution 0.05 M: Dissolve 10.505 gm of citric acid in 100ml water. Sodium citrate solution 0.05 M: Dissolve 14.705 gm of sodium citrate in 100ml water. 46.5 ml of citric acid with 3.5ml of sodium citrate solution and up to 100ml water and filtered through Millipore 0.4micron filter, adjust the PH-5.2 by using KOH.

Preparation of mobile phase

Prepare a mixture of 300 volumes of buffer preparation and 700 volumes of methanol, and Sonicate for 10min.

Diluent: Mobile phase

Chromatographic condition:

The optimized chromatographic conditions of the developed method as shown in table no-1.

Table 1: optimized chromatographic condition

Mobile phase	Methanol: Citrate buffer at PH-5.2 with potassium hydroxide[70:30 v/v]
Stationary phase	Grace smart RP-18 Column dimension ID : 250× 4.6 mm,5µ
Wavelength	254 nm
Run time	10min
Injector	Rheodyne 50µl
Flow rate	1.0 ml per min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of standard stock solution

Weigh accurately about 100mg of Ganciclovir pure drug and then transferred into 100ml volumetric flask and diluted with diluent up to the mark and sonicated for 5 min to dissolve it completely (stock solution-1). From the above solution pipette out 10ml into 100ml volumetric flask and made up to the mark with diluent (stock solution-2),from this solution pipette out 1, 2, 3 , 4, 5, and 6ml into 10ml individual volumetric flask and add diluent up to the mark , this gives 10, 20, 30, 40, 50, 60 µg/ml concentrations.

Preparation of sample solution:

Ten capsules were weighed and powdered, the capsule powder equivalent to 100mg of Ganciclovir was transferred into100ml volumetric flask then it was diluted with diluent and made upto mark

and the solution was filtered through Millipore filter 0.4 micron. From this pipette out 10 ml in a 100ml volumetric flask and make up the volume up to the mark with diluent. From this solution pipette out 1.5ml in 10ml volumetric flask and make up the volume with diluent, this gives 15µg/ml concentrations.

Flow rate selection

Different flow rates in between 0.50 to 1.50 ml /min were studied. A flow rate of 1.0 ml /min gave an optimal signal to noise ratio with a reasonable separation time.

RESULTS AND DISCUSSION

System suitability

20µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N), tailing factor, retention time (RT) were determined. The values of system suitability parameters were shown in Table 2, it indicates good performance of system (Figure 2).

Table 2: System suitability parameters

System suitability parameters	Results
Retention time	2.982
Area	1097125.000
Number of Theoretical plate	5457.336
Tailing factor	1.340

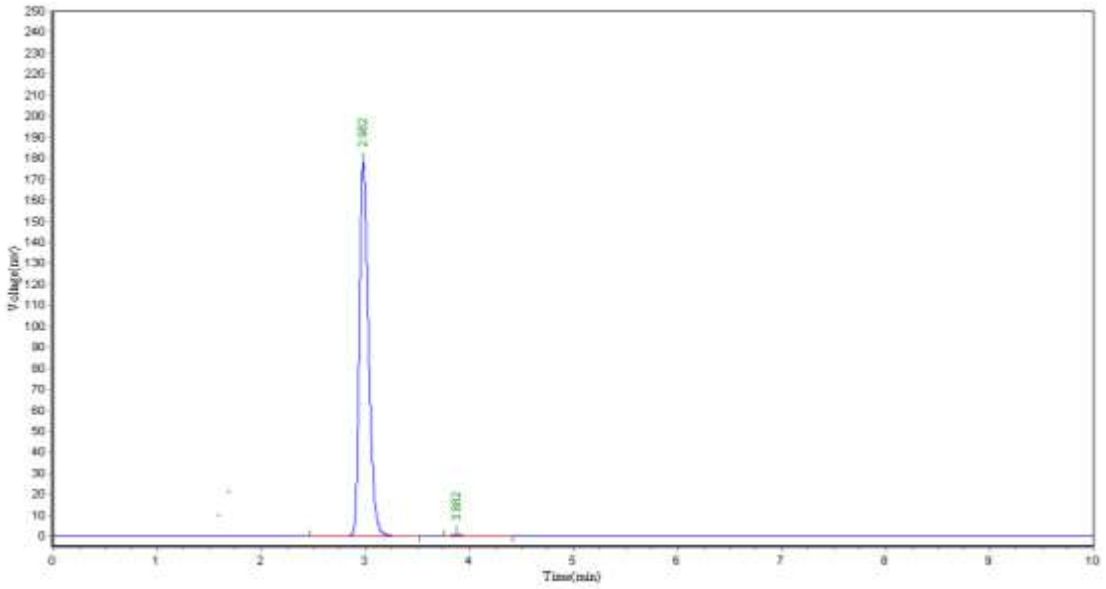

Figure 2: Chromatogram of Ganciclovir

Method validation

The method is validated according to the ICH guidelines[13-15].

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Ganciclovir from excipients. There is no interference of excipient peak on the peak of Ganciclovir indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Figure 3 and showed linearity over a concentration range of 10-60 µg/ml shown in Table 3, along with regression parameters in Table 4. Each calibration was injected three times. The calibration curve was performed in triplicate

Table 3: Linearity data for Ganciclovir

Sl.no	Concentration(mcg/ml)	Peak Area
1	0	0
2	10	3859981.198
3	20	751833.354
4	30	1099698.79
5	40	1481861.5
6	50	1798362.99
7	60	2126004.125

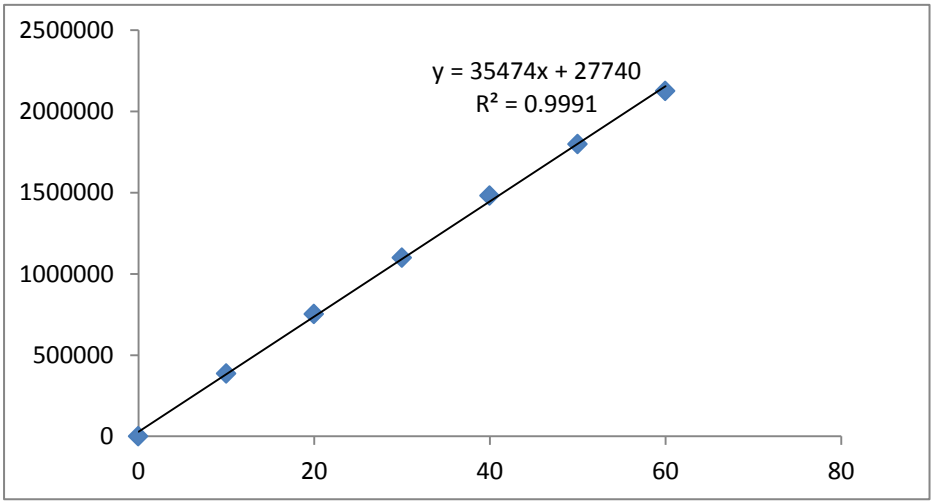

Figure 3: Calibration curve for Ganciclovir

Table 4: Regression parameters table for Ganciclovir

Regression	Parameter Ganciclovir
Regression Equation*	Y=35474x+27740
Slope (b)	35474
Intercept (a)	27740
Correlation Coefficient (r2)	0.999

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. Intra-day precision was determined by analyzing Ganciclovir for six times in the same day at wavelength range 254 nm. Inter-day precision was determined by analyzing the drug daily once for six days at wavelength range 254 nm. The results were shown in terms of %RSD were within the limits, shown in Table 5.

Table 5: Results of Precision studies

Precision	Intra-day	Inter-day		
		Day-1	Day-2	Day-3
Mean area	1099699	108229	1122839	1096181
Standard deviation	556.711	2025.04	11769.12	7671.554
%RSD	0.0506	1.871	1.0481	0.6998

Each determination is average of six replicates, RSD indicates relative standard deviation

Accuracy

Accuracy (recovery) is the closeness of the test results obtained by the method to the true value. That was obtained by spiking 80, 100 and 120% of Ganciclovir working standard conc., in which the amount of marketed formulation was kept constant and amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery

Table 6: Results of Accuracy studies

% Spiked levels	Amount of Formulated drug added In µg/ml	Amount of pure drug added in µg/ml	Amount found In µg/ml	% recovery	Statistical parameters
80	15	9	24.2	100.8	Mean =101.23
	15	9	24.6	102.5	SD=1.11504
	15	9	24.1	100.4	%RSD=1.101
100	15	15	29.4	98.22	Mean =99.76
	15	15	30.6	102.0	SD=1.9848
	15	15	29.71	99.06	%RSD=1.989
120	15	21	36.5	100.3	Mean =100.36
	15	21	35.8	99.4	SD=1.001
	15	21	36.51	101.4	%RSD= 0.997

Robustness

Robustness was carried by varying two parameters deliberately from the optimized chromatographic conditions like mobile phase composition and P^H . The %RSD was found to be <2, shown in Table 7.

Table 7: Robustness results of Ganciclovir

Parameters	pH and mobile phase 5.5 69:31	pH and mobile phase 5.0 71:29
Mean area	1108287.166	1103734.78
Standard deviation	19790.66	6858.5
%RSD	1.755	0.621

Ruggedness

The Ruggedness was determined by using the data obtained by the analysis performed by two between different analysts. The value of %RSD was found to be < 2, showed ruggedness of developed analytical method. The values were shown in Table-8.

Table 8: Ruggedness results for Ganciclovir

Analyst	Analyst-1	Analyst-2
Mean area	1099698.79	1096180.5
Standard deviation	5556.711	7671.554
%RSD	0.5052	0.6998

RSD indicates relative standard deviation

Limit of detection and Limit of quantitation

The LOD and LOQ were calculated by using the slope and SD of response (intercept). LOD and LOQ values of Ganciclovir were found to be 0.246µg/ml and 0.745µg/ml.

CONCLUSION

Thus, the developed method was found to novel, simple, accurate, precise, selective and sensitive for the routine estimation of Ganciclovir in bulk and capsule dosage form.

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