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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF GANCICLOVIR IN BULK DRUG AND ITS FORMULATIONS.

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ABSTRACT

A simple, precise, rapid and reproducible reverse phase high performance liquid chromatographic method has been developed and validated for quantitative estimation of Ganciclovir (GCV) in bulk drug and its formulations using Acyclovir as an internal standard (IS). The chromatographic separation was achieved by using column oyster (250 x 4.6mm, 5µm) mobile phase consist of combination of triflouro acetic acid buffer and methanol at pH 2.5 in the ratio of 80:20 respectively and was pumped at 1.0 mL/min and the injection volume was 10 µL. The detection was carried out at 254 nm. Retention times were 5.823 and 7.107 min for GCV and Acyclovir (IS) respectively. Linearity of method was 12-72 µg/mL, the correlation coefficient was found to be 0.9987. The separation was performed at temperature of 30^0C. The method was validated according to ICH guidelines. Due to its simplicity, rapidness, high precision and accuracy the proposed HPLC method may be used for the determination of GCV in quality control samples and its formulations with out interference of excipients.

KEY WORDS

Ganciclovir (GCV), Acyclovir, Internal standard, RP-HPLC.

INTRODUCTION

Ganciclovir (GCV)[1-4] is chemically 2-amino-1,9-[{2-hydroxy-1-(hydroxymethyl) ethoxy} methyl]-6-H-purine-6-H-one (Fig A), is a synthetic nucleoside analogue closely related to Acyclovir (ACV, Fig B). 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. It is official in Martindale, Merck Index, USP. Literature survey reveals that few High performance liquid chromatographic (HPLC) methods have been described for analysis of GCV in serum and human plasma. Those methods include ion-pairing agents[5,6], gradient elution[7], amperometric detection[8], precolumn flurosecence derivatization[9], electrochemical detection and ion exchange chromatography[10] to determine GCV. The

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aim of this study is to develop a simple, sensitive and precise analytical method for the determination of GCV, incorporating an internal standard (ACV), which can be applied for the determination of GCV concentration in bulk drug and its pharmaceutical formulation.

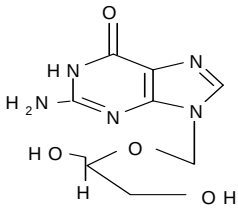

Fig. A: Ganciclovir.

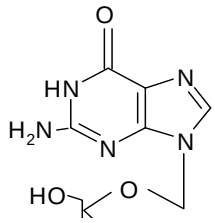

Fig. B: Acyclovir.

EXPERIMENTAL

Equipment

Quantitative HPLC determination is performed on Shimazdzu prominence isocratic HPLC system with LC-20 AT pump, SPD-20A detector, chromatogram were analyzed by using CFR software.

Chemical and reagents

Pure GCV and ACV were obtained as a gift sample from Ranbaxy (super speciallity) Pvt Ltd. Himachal Pradesh. Methanol was of HPLC grade and purchased from Qualigens, Mumbai and other buffer salts were of analytical grade. HPLC grade water was used for the preparation of buffer. Buffer solution was prepared and filtered through 0.45 µm membrane filter. Formulations of GCV used for the study are Ganquard (Ranbaxy Pvt. Ltd) and Natclovir (Natco Pharm Ltd) capsules both containing 250 mg were procured from local market.

Preparation of mobile phase

The content of the mobile phase was prepared from filtered and degassed mixture of trifuoro acetic acid (i.e. prepared by diluting 1 mL of trifluoro acetic acid in 1000 mL of distilled water and pH was adjusted to 2.5) and methanol in the ratio of 80:20 v/v.

Preparation of diluents

Measure accurately 1 mL of trifluoro acetic acid was dissolved in 1000 mL of water. Degas the buffer solution through 0.45 µm membrane filter.

Chromatographic Condition

Separation was performed on reverse phase oyster (250 x 4.6, 5µm particle size) column. Mobile phase consists of mixture of trifluoro acetic acid (prepared by diluting 1 mL of trifloro acetic acid in 1000 mL of distilled water) and methanol (80 : 20). Injection volume of 10 µL was used. Mobile phase was filtered before use through 0. 45 µm membrane filter and degassed with helium purge for 30 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.0 mL/min and wavelength was set to 254 nm. The column temperature was set at 30^0C.

Preparation of bulk drug and internal standard solution

Stock solutions (1mg/mL) of GCV and internal standard ACV were prepared by dissolving 100 mg

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of each in 100 mL volumetric flasks containing 50 mL of diluent and sonicated for about 15 min and then made upto volume with diluent. Working standard of GCV and internal standard were prepared by suitably diluting the stock solution with diluent. Subsequent dilutions of this solution were made with diluent to get concentration of 12 - 72 µg/mL.

Preparation of sample solution

The powder of the sample capsule claimed to contain 250 mg of GCV. The accurate quantity equivalent to 100 mg of active ingredient was extracted with diluent (3 x 15 mL) and filtered through 0.45 µm membrane filter, followed by adding diluent upto 100 mL to get the stock solution of 1 mg/mL. This solution was further diluted stepwise with diluent as under the preparation of standard solution to get the concentration required.

Assay procedure

The column was equilibrated for atleast 30 min, with the mobile phase flowing through the system with a flow rate of 1.0 mL/min. Detector was set at wavelength of 254 nm. Ten sets of the drug solutions were prepared in diluents containing GCV at a concentration range of 12–72 µg/mL along with a fixed concentration of internal standard (ACV, 30 µg/mL). Then 10 µL of each standard and sample solution were injected for six times separately. The retention time of GCV in bulk drug and its pharmaceutical formulation is 5.980 and 5.851 and the retention time of internal standard Acyclovir is 7.132 and 6.964 respectively. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of GCV in capsule dosage form.

ANALYTICAL VALIDATION

Validation of developed analytical method was performed as per ICH guideline over the specificity, accuracy, repeatability, precision, linearity, limit of detection, limit of quantization and robustness.

RESULTS AND DISSCUSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of GCV in bulk samples or its pharmaceutical dosage form. To achieve sharp peaks with good resolution, under isocratic conditions, mixtures of triflouroacetic acid and methanol in different combinations were tested as mobile phase on C_{18} stationary phase. The proposed method was proved to be most suitable for all combinations, since the chromatographic peaks were better defined and resolved.

The internal standard utilized in this method is ACV, a chemical analoge of GCV. This molecule is a good choice because of its solubility in mobile phase and its chromatographic and light absorption properties, which are similar to GCV.The retention time of GCV in bulk drug and its pharmaceutical formulation is 5.980 and 5.851 and the retention time of internal standard ACV is 7.132 and 6.964 respectively. Each of the sample was injected six times and the same retention time were observed in all cases. The peak area of different concentration set up as above was calculated. The peak area for the solution was reproducible as indicated by low coefficient of variation (0.208). A good linear relationship (r = 0.9987) was observed between the concentrations of

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GCV and respective peak areas. The calibration graph was found to be Y = -1246.1539X+3208.3876 where Y is the peak area and X is the concentration of GCV in the range of 12-72 µg/mL, when GCV solution containing 30 µg/mL, 60 µg/mL were analyzed by the proposed RP-HPLC method for finding out intra and inter-day variations. A low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The drug content in the capsules was quantized using the proposed analytical method. The mean content of GCV in two different brands of capsule dosage forms is shown in Table 3. The amount of GCV from the pre-analyzed sample containing known amounts of the drug is shown in Table 4. About 99.76±0.14 and 99.72±0.09 GCV could be recovered from the pre-analyzed sample indicating the high accuracy of the proposed HPLC method. The absence of additional peaks indicates no interference of the excipients used in the capsules. The capsules were found to contain 99.48 to 99.51 of the labeled amount. Less than 1% CV indicates reproducibility of the assay of GCV in the capsule dosage form. The proposed RP-HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

Table 1.

Optical Characteristics and Precision

Parameter	GCV	ACV
Retention time (t) min	5.813	7.107
Theoretical plates (n)	10324.973	11968.527
Plates per meter(N)	68833.154	47874.110
Height equivalent to theoretical plate (HETP)	14.528	20.888
Tailing factor	1.114	1.085
Relative retention	0.645	
Resolution factor	5.322	
Linearity range (µg/mL)	12 - 72	
LOD (µg mL^{-1})	1.51	
LOQ (µg mL^{-1})	4.57	
Regression equation (Y=bC+a)		
Slope (b)	-1246.1539	
Standard deviation of slope (S_b)	3.494805	
Intercept (a)	3208.3876	
Standard deviation of intercept (S_a)	0.752770	
Standard error of estimation (Se)	0.519	
Correlation coefficient (r)	0.9987	
Relative standard deviation	0.208	

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Range of errors**	
Confidence limits with 0.05 level (±)	0.94
Confidence limits with 0.01 level (±)	0.88
% Error in bulk Samples***	0.512

**Y=bC+a, where C is the concentration of GCV in µg/ml and Y is the absorbance at the respective maximum absorbency, **Average of eight determination, ***Average of three determination*

Table 2

Inter and intra day precision for GCV assay in pharmaceutical dosage form by proposed method.

Concentration of GCV (µg/mL)	Observed concentration of GCV (µg/mL)			
	Intra day		Inter day	
	Mean	% CV	Mean	% CV
30	100591.7665	0.07923	100812.0834	0.08165
60	201183.833	0.15847	201624.1667	0.1233

*Average of five determinations

Table 3

Assay results of GCV in pharmaceutical formulations

Sample	Labelled amount (mg)	Amount found*±SD	Reference method (UV)	% recovery± RSD
Capsule C_1	250	248.72 ± 0.50 t = 0.225 F = 0.889	249.86 ± 0.53	99.48
Capsule C_2	250	248.54 ± 0.63 t = 0.63 F = 1.355	248.74 ± 0.44	99.51

C_1 = Ganquard 250 mg (Ranbaxy, Superspeciality);
C_2 = Natclovir 250 mg (Natco pharma)

**Average ± standard deviation of eight determinations, the t and F-values refer to comparison of the proposed method with reference method. Theoretical values at 95% confidence limits t=2.365 and F=4.88.*

Table 4

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Recovery studies of GCV in pharmaceutical formulations

Pharmaceutical formulations	Amount (µg/mL)		% Recovery ± R.S.D.
	Taken + added	Found* ± SD	
Capsule I	30 + 30	59.25 ± 0.02	98.76 ± 0.14
Capsule II	60 + 60	119.66 ± 0.04	99.72 ± 0.09

*Average of six determinations

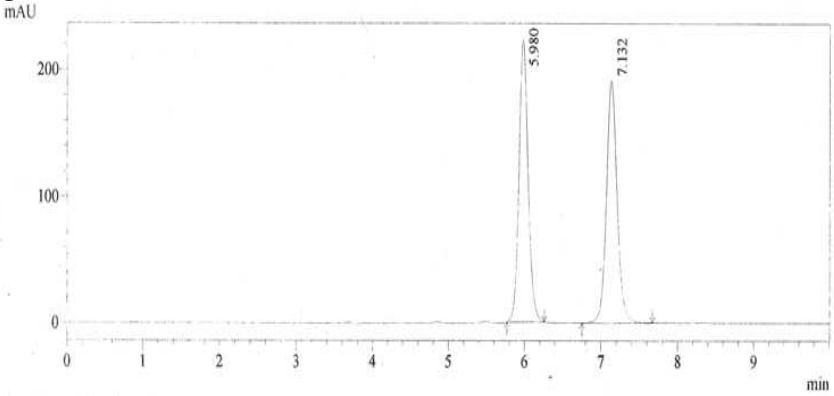

Figure 1.
Model chromatogram for GCV in bulk drug sample (RT 5.980) with internal standard ACV (RT 7.132)

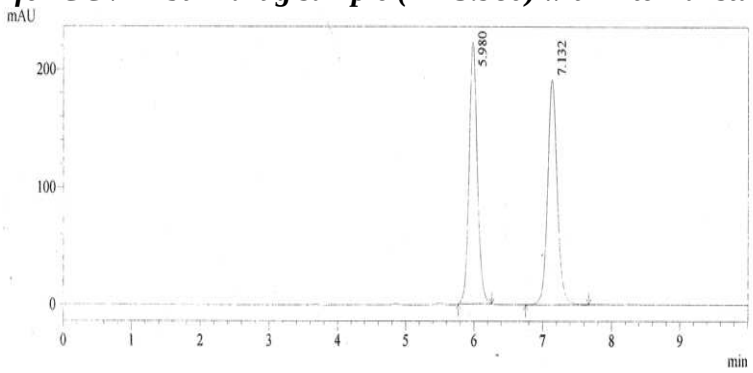

Figure 2.
Chromatogram for GCV in Pharmaceutical formulation (RT 5.851) with internal standard ACV (RT 6.964)

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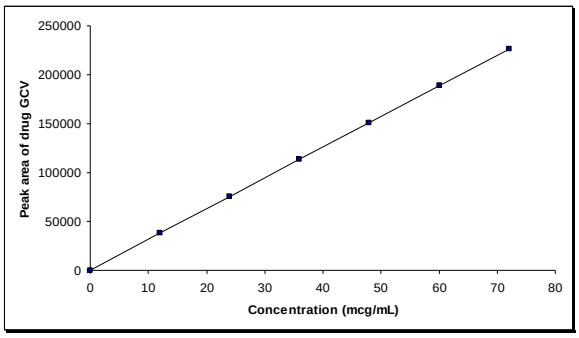

Figure 3.
Standard calibration graph of GCV (12-72 µg/mL).

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