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Spectrophotometric Estimation of Ganciclovir in Bulk Drug and its Formulation

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ABSTRACT: Ganciclovir is an acyclic guanosine analog used in the treatment of AIDS. In present work, three new simple, sensitive, accurate and economical spectrophotometric methods have been developed for the estimation of Ganciclovir in bulk drug and its pharmaceutical formulation. Method A is the first order derivative spectroscopy method adopted to eliminate spectral interference, in which derivative amplitude was measured at 238 nm with n=1. Method B is based on calculation of area under curve for analysis of ganciclovir in wavelength range of 245-255 nm. The drug follows Beer's law in the concentration range of 5-25 µg/mL in both methods. In method C is based on reduction of the hetropoly acid, phosphomolybdotungstic acid, the well known Folin-Ciocalteu reagent in presence of alkali to form intense blue color chromogen exhibiting absorption maximum at 764.7nm and it obeyed Beer's law in the concentration range of 50-250 µg/mL. Results of the analysis were validated for accuracy, precision, LOD, LOQ and recovery studies and were found to be satisfactory. The proposed methods are simple, rapid and suitable for the routine quality control application.

Key words: Ganciclovir, UV spectrophotometry, Derivative spectroscopy, Area under curve, Folin- ciocalteau reagent, Sodium hydroxide.

INTRODUCTION

Ganciclovir[1-4] is chemically 2-amino-1,9-[{2-hydroxy-1-(hydroxymethyl) ethoxy} methyl]-6-H-purine-6-H-one. Ganciclovir is an acyclic guanosine analog. It is used in the treatment of cytomegalovirus (CMV) infection in AIDS patients. It is also exhibit 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 Ganciclovir 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 Ganciclovir. There is no spectrophotometric methods reported so far for the estimation of Ganciclovir using spectrophotometry in bulk drug or its formulations. Hence the present work deals with the spectrophotometric estimation of Ganciclovir using first order derivative spectra and calculation of area under curve (AUC) for analysis of Ganciclovir and one visible spectrophotometric method by Folin-Ciocalteu reagent in presence of alkali. The above methods are simple, sensitive, accurate and precise, can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulation.

EXPERIMENTAL METHODS

Instruments

All spectral measurements were made on Shimadzu UV 1700 UV/VIS spectrophotometer was used with 1cm matched quartz cells.

Reagents and chemicals

FC reagent and ferric chloride of AR grade were purchased from SD Fine Chemicals Ltd., Mumbai, dissolved and diluted with distilled water were used as solvents. Pure Ganciclovir was obtained as a gift sample from Ranbaxy (super speciallity) Pvt Ltd., Himachal Pradesh.

Preparation of standard and sample:

Accurately weighed 100 mg of Ganciclovir (bulk drug) was dissolved in sufficient quantity of distilled water and the volume was made upto 100 mL with distilled water (1000 µg/mL). Further dilution was made to give stock solution 100µg/mL.

Method A:

Fresh aliquots from standard stock solution were pipetted out and suitably diluted with distilled water to get the final concentration 2.5- 25 µg/mL. The solutions were scanned in the spectrum mode for 400 to 200 nm wavelength range and the first derivative spectra were obtained at n=1 as sharp peak obtained as 238 nm. The absorbance difference at n=1 ($dA/d\lambda$) was calculated by inbuilt software of the instrument which is directly proportional to concentration of standard solution. A calibration curve was plotted taking the absorbance difference ($dA/d\lambda$) against the concentration of the standard solution. The method was applied for the sample solution of known concentration and was found to be satisfactory for the analysis of capsule formulation.

Method B:

Area under curve method is applicable when there is no sharp or when broad spectra are obtained. It involves the calculation of integrated values of absorbance with respect to the wavelength between two selected wavelengths, $\lambda 1$ and $\lambda 2$. Area calculation processing item calculation the area bound by the curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area is selected on the basis of repeated observations so as to get linearity between the area under curve and concentration. Fresh aliquots from standard stock solution were pipetted out and suitably diluted with distilled water to get the final concentration 2.5- 25 µg/mL. The solutions were scanned in the spectrum mode for 400 to 200 nm wavelength range and area under curve was measured at 245-255 nm. Calibration curve was plotted as concentration against AUC.

Method C:

Fresh aliquots of Ganciclovir ranging from 0.5 to 2.5 µg/mL (1 mL-1000 µg/mL) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 50 to 250 µg/mL. To each flask 1.5 mL of Folin- ciocalteau reagent (1 N) solution and 1.5 mL of sodium hydroxide (1 N) were added. The solutions were kept aside for 10 min and made upto the mark with distilled water. The absorbance of blue colored chromogen was measured at 764.7 nm against the reagent blank.

Assay determination:

For the estimation of Ganciclovir in capsule formulation by all the three methods, 20 capsule of each brand were weighed and triturated to fine powder. Capsule powder equivalent to 100mg of Ganciclovir was weighed, dissolved and further diluted with sufficient quantity of distilled water. This was then filtered through whatmann's filter paper No. 41 to get the stock solution of concentration 100 µg/mL.

For the method A, analysis of Ganciclovir in both capsule formulation T_1 and T_2 was done using various sample solution at 238 nm against reagent blank in quantization mode for five times. In method B, the sample solutions were scanned in the spectrum mode and AUC calculation were done in the wavelength range of 245-255 nm for the capsule formulation. In method C, the sample solutions were scanned in the spectrum mode in the wavelength range of 650-790 nm for the capsule formulation and beer's law was obeyed in the range of 50 to 250 µg/mL. The amount of Ganciclovir present in the sample solution was computed from its calibration curve.

RESULTS AND DISCUSSION:

The optical characteristics such as Beers law limit, sandell's sensitivity, molar extinction coefficient, percent relative standard deviation (calculation from eight measurements containing ¾ th of the amount of the upper Beers law limit) were calculated. Regression Characteristics like slope, intercept, correlation coefficient and percentage range of errors (0.05 and 0.01 confidence limits), LOD, LOQ, Error's in bulk sample and standard error of estimation obtained from different concentration and the results will be summarized in Table 1

Commercial formulation of Ganciclovir was successfully analyzed and results were calculated. To evaluate validity and reproducibility of the methods, fixed amounts of drug were added to the preanalyzed formulation. These results of percentage recovery were summarized in Table 2. There was no interference of additives or excipients in proposed analytical methods. The methods reported were found to be simple, sensitive, accurate and precise and can be used for the routine quality control of this drug in bulk as well as in pharmaceutical formulations.

CONCLUSION

As the drug Ganciclovir showed a broad spectrum, the derivative spectroscopy method applied has the advantage that it locates the hidden peak in normal spectrum when spectrum is not sharp and it also eliminates the interference caused by the excipients and the degradation product present, if any in the formulation. The AUC method is also advantageous as it is applicable to the drug which shows the spectra without a sharp peak. The visible spectrophotometric methods can be used for the estimation of drug in bulk as well as in pharmaceutical formulations. Hence these methods can be useful in the routine analysis.

Table 1: Optical Characteristics and Precision

Parameters	Method A	Method B	Method C
λ_{max} (nm)	238	245-255	764.7
Beer's law limits (µg/ml) (c)	2.5 - 25	2.5 - 25	50 - 250
Molar absorptivity (lit/mol^{-1} cm^{-1})	4.957 x 10^5	1.054 x 10^3	1.25x10^2
Limit of Detection (LOD/ mcgml^{-1})	2.25	0.014	2.12
Limit of Quantification (LOQ/ mcgml^{-1})	6.83	0.043	6.45
Sandell's sensitivity (µg/ml/0.001 abs units)	0.0012	0.005	0.0029
Regression equation (Y*) Slope (b)	1.9437 x 10^{-3}	7.1905 x 10^{-1}	3.1043x10^{-3}
Intercept (a)	4.466 x 10^4	3.3609 x 10^{-1}	1.287x10^2
Standard error of estimation (Se)	0.0046	0.011	0.0109
Correlation coefficient (r)	0.9976	0.9997	0.9999
% RSD	0.467	0.0492	0.640
Range of errors** Confidence limits with 0.05 level	0.0016	0.0038	0.0026
Confidence limits with 0.01 level	0.0011	0.0026	0.00384
% Error in bulk Samples***	0.432	0.220	0.456

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

Table 2: Assay and Recovery of Ganciclovir in pharmaceutical Dosage Form

Pharmaceutical dosage form	Labeled Amount	Amount found by proposed methods (mg)			Reference method (UV in 0.1N HCl)	Recovery of proposed methods (%)		
		Method A	Method B	Method C		Method A	Method B	Method C
T_1 (Ganquard)	250 mg	248.92	249.45	248.66	249.86	99.56	99.78	99.46
T_2 (Natclovir)	250 mg	249.05	248.38	249.36	248.74	99.62	99.35	99.74

T_1, T_2 are capsules from different manufacturers, average of 5 determinations (100 mg of Ganciclovir was added and recovered).

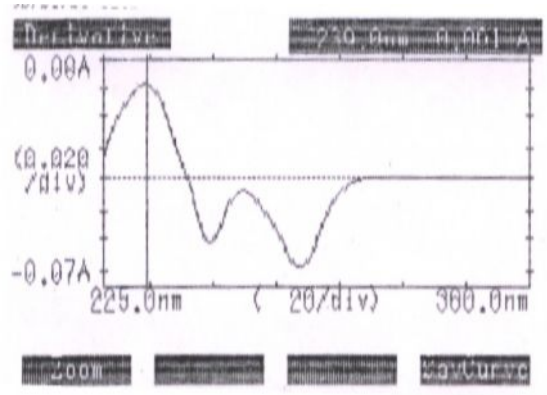

Figure:- Absorbance spectrum for Ganciclovir by first derivative spectroscopy

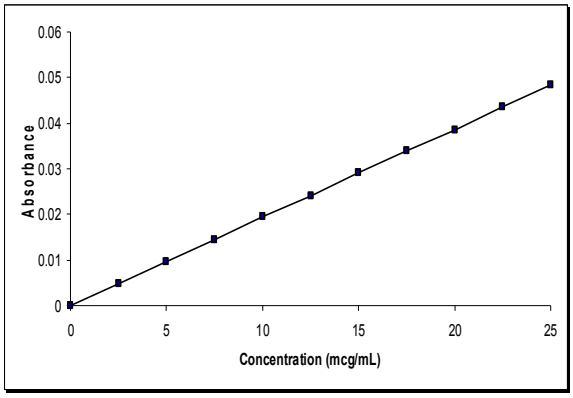

Figure:- Beer's law curve for Ganciclovir at 238 nm

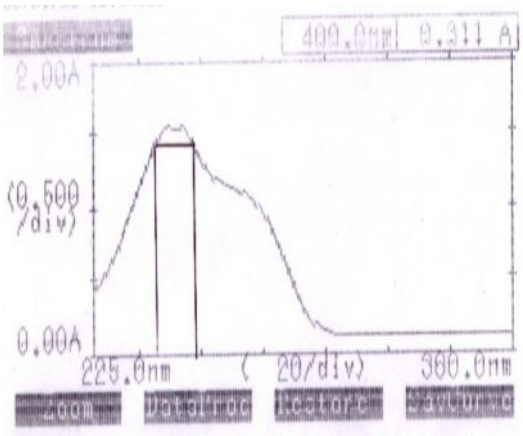

Figure:- Absorbance spectrum for Ganciclovir by Area under curve method

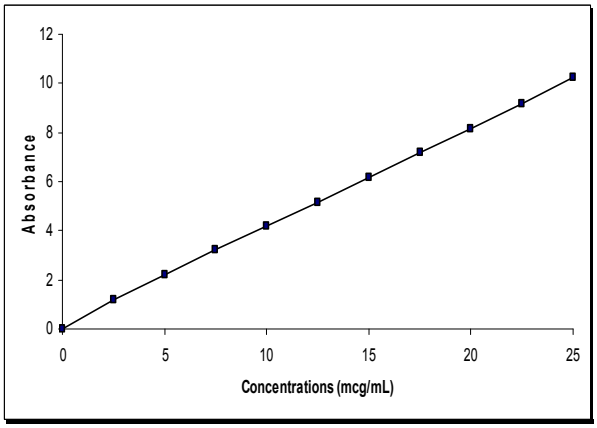

Figure:- Beer's law curve for Ganciclovir at 245-255 nm

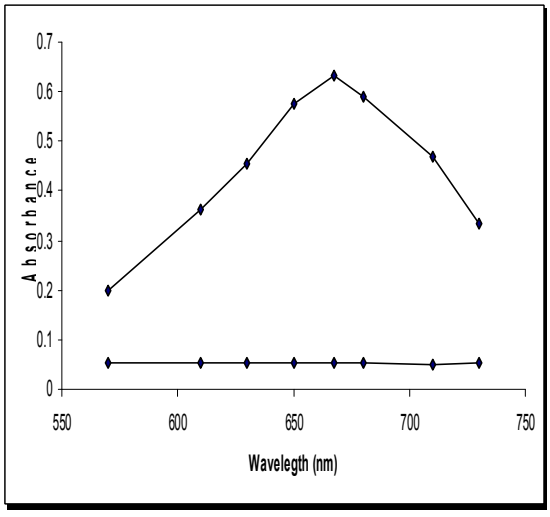

Figure:- Absorbance spectrum for Ganciclovir by FC reagent method

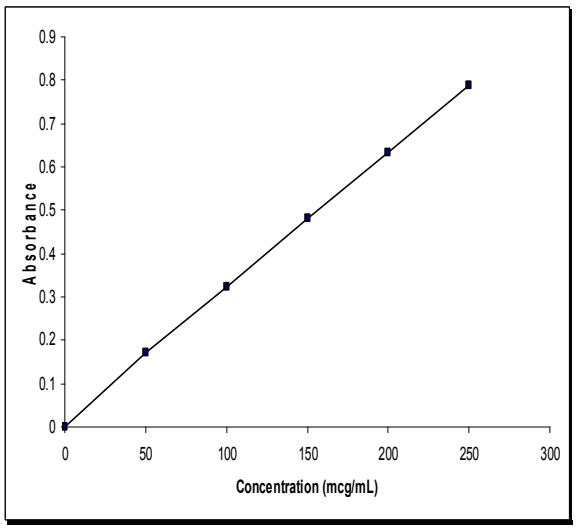

Figure:- Beer's law curve for Ganciclovir at 50-250 µg/mL.

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