International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 282-285, Jan-Mar 2010

VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF GANCICLOVIR BY CONDENSATION AND OXIDATIVE COUPLING REACTIONS

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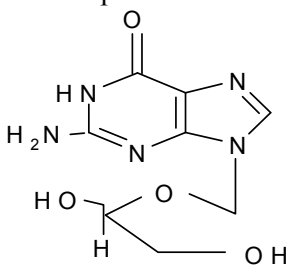
ABSTRACT: Two simple, sensitive, selective, accurate, precise and economical methods (method A and B) have been developed for the quantitative estimation of Ganciclovir in bulk drug and its pharmaceutical formulations. In method A the presence of amino group in Ganciclovir enables the use of condensation reaction with P-dimethylamino cinnmaldehyde (PDAC) the orange red colored complex is due to formation of Schiff's base. Method B is based on oxidation followed by coupling of 3 methyl-2-benzothiazolinone hydrazone (MBTH) with Ganciclovir in presence of ferric chloride to form bluish green color chromogen and exhibiting absorption maximum at 524 nm and 611.8 nm respectively and obeying beers law in the concentration range of 10 – 50 µg/ml and 50 – 250 µg/ml respectively. The results of analysis for both the methods have been validated statistically and by recovery studies, the results of both the methods are compared with those obtained by using UV spectrophotometric methods developed in our laboratory with 0.1 N HCl at 253 nm.

Key words: Ganciclovir, PDAC, MBTH.

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 that requires triphosphorylation for activation prior to inhibiting the viral DNA polymerase. It is used in treatment of cytomegalovirus (CMV) infection in AIDS patients. Ganciclovir exhibit antiviral activity against herpes simplex virus (HSV) and cytomegalovirus (CMV) at relatively low inhibitory concentrations.

Literature survey reveals that few methods like liquid chromatography using pulsed amperometric detection in plasma[5], high performance liquid chromatography (HPLC) with precolumn fluorescence deviation using phenylglyoxal in serum[6]. No spectrophotometric methods reported so far for the estimation of Ganciclovir using visible spectrophotometry in bulk drug or its formulations. Hence the present work deals with the spectrophotometric estimation of Ganciclovir using PDAC reagent in presence of hydrochloric acid in method A & MBTH in presence of $FeCl_3$ in method B.



Ganciclovir

EXPERIMENTAL

All spectral measurements were made on systronics 119 UV/visible spectrophotometer chemicals. All the chemicals and reagents used were of analytical reagent grade.

1) P-dimethylamino cinnmaldehyde (PDAC)
2) 3 methyl-2-benzothiazolinone hydrazone (MBTH)
3) Ferric Chloride
4) Distilled Ethanol
5) Distilled Water
6) Bulk drug, Ganciclovir (Ranbaxy, Superspeciality Ltd)

Preparation of Standard:

Accurately weighed 100 mg of Ganciclovir was dissolved in 40 ml of distilled water and further diluted with sufficient quantity of distilled ethanol (i.e.1000 µg/mL). Further dilution was made with distilled ethanol to get the concentration of 100 µg/mL. In method B distilled water is used as solvent for dissolving the bulk drug.

Preparation of Sample:

For the estimation of Ganciclovir 20 capsule of each brand were weighed and triturate to fine powder. Capsule powder equivalent to 100mg of Ganciclovir was weighed, dissolved in 40 ml of distilled water and further diluted with sufficient quantity of distilled ethanol. This was then filtered through whatman filter paper no. 41 to get the stock solution of concentration 100 µg/mL. Further dilution was made with distilled ethanol to get the concentration of 100 µg/mL. In method B distilled water is used as solvent for dissolving the bulk drug or its formulation.

Method A

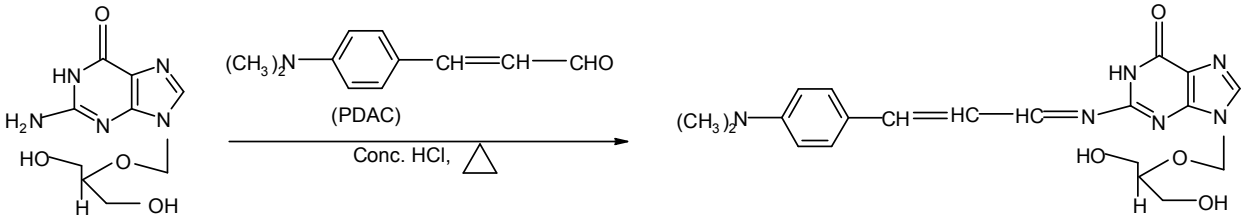
PDAC Method[7,8]

Fresh aliquots of Ganciclovir ranging from 1 to 5 mL (1 ml =100 µg/mL) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 10 to 50 µg/mL. To each flask 1 ml of alcoholic PDAC (0.5%) solution and two drops of conc. HCl were added and heated at 40^0C for 20 min. The solutions were cooled to room temperature and made upto mark with distilled ethanol. The absorbance of orange red colored chromogen was measured at 524 nm against the blank. The amount of Ganciclovir present in the sample solution was computed from its calibration curve.

Method B

MBTH Method[9,10]

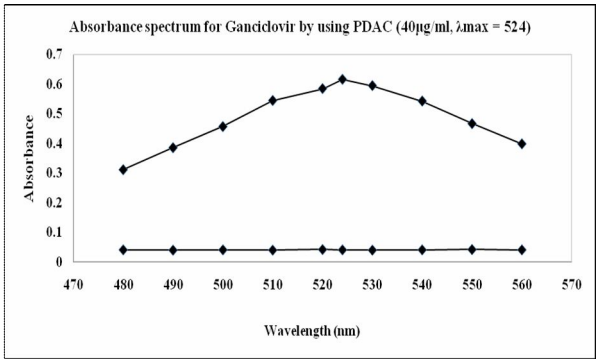
Fresh aliquots of Ganciclovir ranging from 0.5 to 2.5 mL (1 ml-1000µg/mL) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 50 to 250 µg/mL. To each flask 1ml of aqueous Ferric chloride (1%) solution and 1 ml of MBTH reagent (0.5% in distilled water) were added. The solution in each tube were made upto the mark with distilled water. The absorbance of bluish green colored chromogen was measured at 611.6 nm against the blank. The amount of Ganciclovir present in the sample solution was computed from its calibration curve.

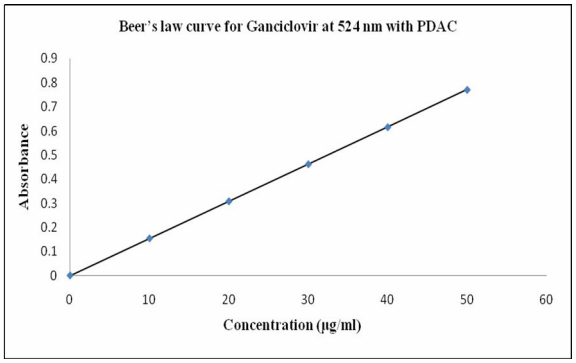


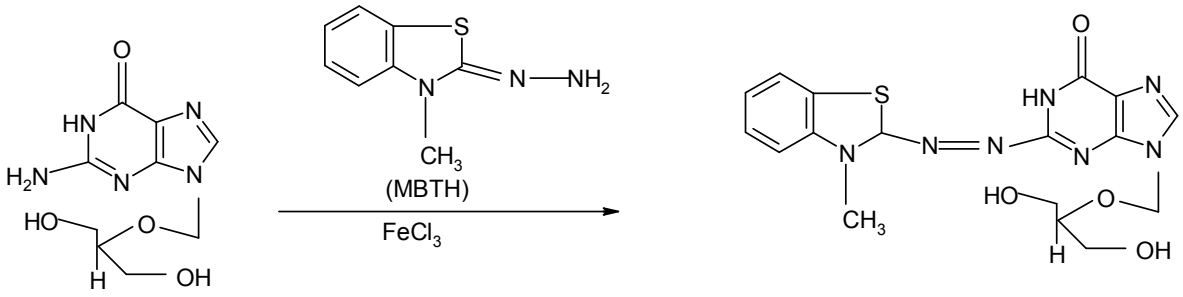
Ganciclovir **Orange red color chromogen**

λ max 524 nm

Method A



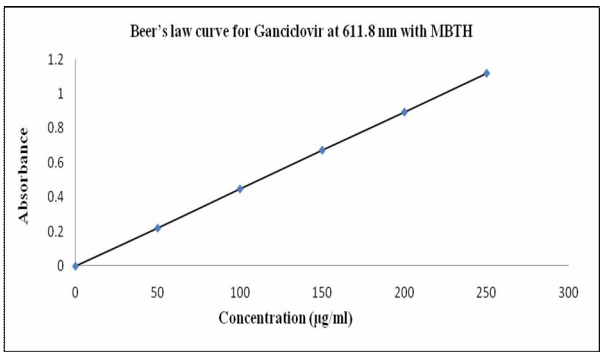




Ganciclovir **Bluish Green color chromogen**

λmax 611.8

Method B



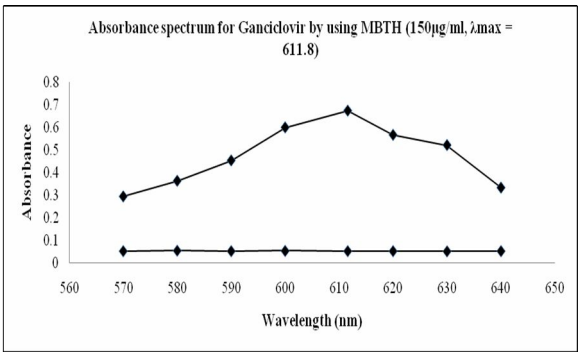


Table 1: Optical Characteristics and Precision

Parameters	**Method-A**	**Method-B**
λ_{max} (nm)	524	611.8
Beer's law limits (µg/ml) (c)	10 -50	50-250
Color	Orange red	Bluish green
Molar absorptivity (lit/mol^{-1} cm^{-1})	1.175 x 10^3	1.447 x 10^3
Limit of Detection (LOD/ mcgml^{-1})	0.425	1.52
Limit of Quantification (LOQ/ mcgml^{-1})	4.60	1.28
Sandell's sensitivity (µg/ml/0.001 abs units)	0.0015	0.0042
Regression equation (Y*)		
Slope (b)	1.541 x 10^{-2}	4.4819 x 10^{-3}
Intercept (a)	-1.999 x 10^{-4}	-4.666 x 10^{-4}
Standard error of estimation (Se)	7.007 x 10^{-4}	7.30 x 10^{-4}
Correlation coefficient (r)	0.9999	0.9999
% RSD	0.429	0.307
Range of errors**		
Confidence limits with 0.05 level	0.0016	0.0017
Confidence limits with 0.01 level	0.0024	0.0025
% Error in bulk Samples***	0.292	0.122

*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	Labelled Amount	Amount found by proposed methods (mg)		Reference method (UV in 0.1N HCl)	Recovery of proposed methods (%)	
		A	B		A	B
T_1 (Ganquard)	250 mg	249.7	248.19	249.86	99.01	98.96
T_2 (Natclovir)	250 mg	248.18	249.27	248.74	99.03	99.44

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

RESULT 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 and summarized in table 1. 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 were calculated and are shown in table 1.

Commercial formulation of Ganciclovir was successfully analyzed by proposed and UV methods and results are presented in table 2. To evaluate validity and reproducibility of the methods, fixed amounts of drug were added to the preanalyzed formulation. These results of percentage recovery are summarized in table 2. There is no interference of additive and exicipients in proposed analytical methods. The proposed spectrophotometric methods for the estimation of Ganciclovir are 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.

ACKNOWLEDGMENT

The authors are thankful to Ranbaxy (super specialty) Ltd. H.P., for providing the drug sample and Head of department, University College of pharmaceutical science, Andhra University, Visakha-patnam and Principal H.K.E Society's college of pharmacy, Gulbarga for providing the facilities to carry out the present work.

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