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Redox-based Spectrophotometric Method for the Determination of Ganciclovir in Bulk and Pharmaceutical Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Ganciclovir is a synthetic analogue of 2′-deoxy- guanosine, used in the treatment of cytomegalovirus infection. The aim of the present investigation was to develop and validate a simple, rapid and sensitive redox-based spectrophotometric method for the quantification of Ganciclovir in pure and in pharmaceutical dosage form.
Methods: It was developed by using 0.1 M HCl as solvent and mixture of ferric chloride and 1,10-phenanthroline as chromogenic reagent. The developed method was optimized for various method conditions and then statistically validated.

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Results: The mixture containing 0.3% w/v of ferric chloride and 0.5% w/v of 1,10-phenanthroline and drug to reagent mole ratio of 0.5:0.5, were identified as optimum for the method, based on the optimization studies. Linearity of the method was found to be 5.0-30.0 µg/mL for Ganciclovir, when the measurement was done at 510.0 nm. The method was proved to be sensitive by its low limit of detection and quantification values i.e., 0.30 and 0.90 µg/mL, respectively. The results of the validation parameters comply with the ICH guidelines. The %assay value 99.2 indicated the successful adaptation of the contemplated method for the pharmaceutical formulation.
Conclusion: The developed method was simple and could be applied in the quality control testing of Ganciclovir in formulation.

Keywords: Ganciclovir; 1,10-phenanthroline; redox-coupling reaction; spectrophotometry.

1. INTRODUCTION

Ganciclovir is chemically known as 2-amino-9-(1,3-dihydroxypropan-2-yloxymethyl)-1H-purin-6-one [1]. It is an antiviral agent used for the prevention of cytomegalovirus disease in bone marrow and solid organ transplant recipients and also used to treat people with weakened immune system [2,3]. The structure of Ganciclovir was shown in Fig. 1.

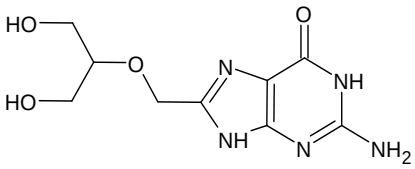

Fig. 1. The chemical structure of Ganciclovir

A rigorous literature search disclosed diverse analytical methods for Ganciclovir in single or in composition with other antiviral agents. Colorimetric methods using Folin-ciocalteu reagent [4], *p*-dimethylaminobenzaldehyde [5,6], Folin regent [5], potassium permanganate [7], UV spectrometric methods using chloroform [8] and 0.1 M HCl [9] as solvents, area under the curve method (AUC) and first order derivative spectroscopy [4], spectrofluorimetry [10], HPLC [11], RP-HPLC [12,13] and bioanalytical methods using HPLC [14] were reported for quantification of Ganciclovir. There were also LC-MS/MS method [15] and liquid chromatography tandem mass spectrometric method using human plasma [16] described for Ganciclovir in combination with other drugs.

1,10-Phenanthroline is a well-known heterocyclic compound. Being a redox indicator, it has potential of forming chelates with metal ions in various redox reactions. Ferric salts played a significant role in spectrophotometric determination of many pharmaceutical substances. Ferric chloride possesses oxidizing property and hence, causes oxidation of analyte with suitable structural features, by itself undergoing reduction. 1,10-Phenanthroline forms complex with the resulting ferrous ions (Fe^{2+}) and the obtained chromogen has absorption maximum in visible region [17,18].

Visible spectrophotometry is regarded as the most convenient method due to ease of instrumentation, measurement and availability of wide variety of reagents [19]. The costly instrument set-up, lack of expertise operators, expensive solvents and tedious extraction procedures limit the use of chromatographic methods. Many of the available spectrophotometric methods for Ganciclovir have narrow linearity range and low sensitivity (Table 1). No simple colorimetric method using Fe^{3+}/1,10-phenanthroline was reported for Ganciclovir to our cognizance. In view of the above remarks, the present method was developed for Ganciclovir using redox-coupling reaction with Fe^{3+}/1,10-phenanthroline. The contemplated method has been tested statistically as per the guidelines of ICH [20].

2. METHODOLOGY

2.1 General

All chemicals and reagents used in the present investigation were of analytical grade. Ganciclovir was kindly provided by Hetero Drugs Pvt. Ltd. and the commercial formulation (Cytovene IV, Genentech, Inc, South San Francisco) was procured from local pharmacy. Double–beam UV–Visible Spectrophotometer 1800 (Shimadzu, Japan) containing spectral bandwidth of 0.1 nm, wavelength accuracy ± 0.1 nm and a pair of matched quartz cells with 1 cm pathlength were used to record the absorbance.

Table 1. Comparison between present and reported spectrophotometric methods

Method/reagent	Solvent - λ_{max} (nm)	Linearity range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Reference
Visible method/Folin-ciocalteu reagent	Water - 764.7	50-250	2.12	6.45	[4]
Visible method/*p*-dimethylaminobenzaldehyde	Water – 401	80-200	80	200	[5]
Visible method/Folin's reagent	Water – 544	4-14	1	12	[5]
Visible method/*p*-dimethylaminobenzaldehyde	0.1 M HCl – 404	10.3 – 25.7	0.23	0.70	[6]
Visible method/potassium permanganate	Water - 610	2-100	0.21	0.72	[7]
UV method	Chloroform – 240	1-4	-	-	[8]
UV method	0.1 M HCl – 255	2-16	0.147	0.445	[9]
UV method – AUC	Water - 245-255	5-25	0.014	0.043	[4]
First order derivative spectroscopy	Water – 238	5-25	2.25	6.83	[4]
Visible method/Fe^{3+}/1,10-phenanthroline	0.1 M HCl - 510.0	5-30	0.30	0.90	Present method

LOD: Limit of detection; LOQ: Limit of quantification

2.1.1 Preparation of ferric chloride reagent (0.3% w/v)

Ferric chloride (0.30 g) was accurately weighed, transferred to a volumetric flask and dissolved in sufficient distilled water to produce 100.0 mL.

2.1.2 Preparation of 1,10-phenanthroline reagent (0.5% w/v)

The accurately weighed 1,10-phenanthroline (0.50 g) was dissolved in sufficient methanol (in a volumetric flask) to make 100.0 mL.

2.1.3 Preparation of Ganciclovir standard stock solution

The standard stock solution (1000.0 µg/mL) of Ganciclovir was produced by solubilizing accurately weighed Ganciclovir (10.00 mg) in 10.0 mL of distilled water. From this 100.0 and 10.0 µg/mL solutions were made by serial dilution and were utilized in the method development.

2.2 Analysis of Ganciclovir

Aliquots of about 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of standard solution of Ganciclovir (100.0 µg/mL) were transferred to a set of 10 mL volumetric flasks. To this ferric chloride (0.3% w/v, 2 mL), 1,10-phenanthroline solution (0.5% w/v, 2 mL) were added and agitated. They were kept aside for 15 min and finally the volume was produced up to the mark by adding 0.1 M HCl to produce final concentration in the range of 5.0 - 30.0 µg/mL of Ganciclovir. Appropriate blank solutions were analyzed by omitting the analyte. The absorbance of six repeated solutions of the same concentration was recorded at 510.0 nm.

2.3 Optimization of Method Conditions

The effect of method conditions, such as concentration of the reagents (ferric chloride and 1,10-phenanthroline), time for color development and mole ratio of the reactants were studied through optimization and the details were provided in the subsequent sections.

2.4 Validation of the Method

The proposed methodology was authenticated for a few validation variables as stated in ICH specifications.

2.4.1 Linearity

The linearity of the method was checked (n=6) over the concentration range of 5.0 – 30.0 µg/mL. Calibration curve showing the relationship between absorbance and concentration of Ganciclovir was plotted. Variables such as, slope, intercept and correlation coefficient were computed from the same.

2.4.2 Accuracy

The accuracy of the procedure was established using different levels of standard solutions (80, 100 and 120%), which were spiked to pre-quantified samples and were tested by

suggested method. The recovery was verified by analyzing analyte in triplicate preparations at each concentration level.

2.4.3 Precision

Solutions comprising of 5.0, 15.0 and 30.0 µg/mL of Ganciclovir were subjected to the proposed colorimetric procedure to check its repeatability. Same concentrations were also checked in triplicate on three different days over a week for establishing intermediate precision of the method. The results of both were expressed as percentage relative standard deviation (%RSD).

2.4.4 Sensitivity and robustness

Sensitivity of the colorimetric procedure was indicated by limit of detection and limit of quantification values. The LOD was calculated by 3.3 multiplied by the ratio of standard deviation and slope. The LOQ was calculated by 10 multiplied by the ratio of standard deviation and slope. Molar absorptivity of the analyte and using that Sandell's sensitivity of the method were calculated. The effect of small changes in the concentration of ferric chloride and 1,10-phenanthroline on the UV absorption of the resulting chromogen was also investigated to ensure robustness of the methodology.

2.5 Assay of Ganciclovir

Vials containing Ganciclovir powder for injection formulation (Cytovene IV) were utilized for the analysis. Powder containing Ganciclovir equivalent to 500.0 mg was weighed with accuracy and suspended in 100.0 mL of distilled water. The contents were mixed well and passed via Whatmann's filter paper (No. 41). Clear filtrate (2 mL) was shifted to a volumetric flask containing ferric chloride solution (0.3% w/v, 2 mL) and 1,10-phenanthroline reagent (0.5% w/v, 2 mL). The solutions were diluted up to 10.0 mL with 0.1 M HCl. The absorbance of the resulting chromogen was determined at 510.0 nm using appropriate blank.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

The colorimetric method proposed was based on a redox-coupling reaction between Ganciclovir and Fe^{3+}/1,10-phenanthroline. The probable reaction mechanism was illustrated in Fig. 2. The ferric chloride causes oxidation of Ganciclovir and itself reduced to ferrous form (Fe^{2+}). The resulting ions of ferrous formed complex with 1,10-phenanthroline and produced an orange colored ferrous-1,10-phenanthroline complex which has λ_{max} at 510.0 nm (Fig. 3).

Ferric chloride and 1,10-phenanthroline were mixed in variable concentrations to optimize the method by considering each variable at a time. The effect of a fixed volume (2 mL) of ferric chloride and 1,10-phenanthroline at the concentration range of 0.1-1.0% w/v was deliberated, by maintaining Ganciclovir concentration at 30.0 µg/mL. The relevant particulars were shown in Fig. 4 and 5. The absorbance was found to be maximum, when the methodology was run with 0.3%w/v of ferric chloride and 0.5%w/v of 1,10-phenanthroline. Hence, the same were chosen optimum for the later studies.

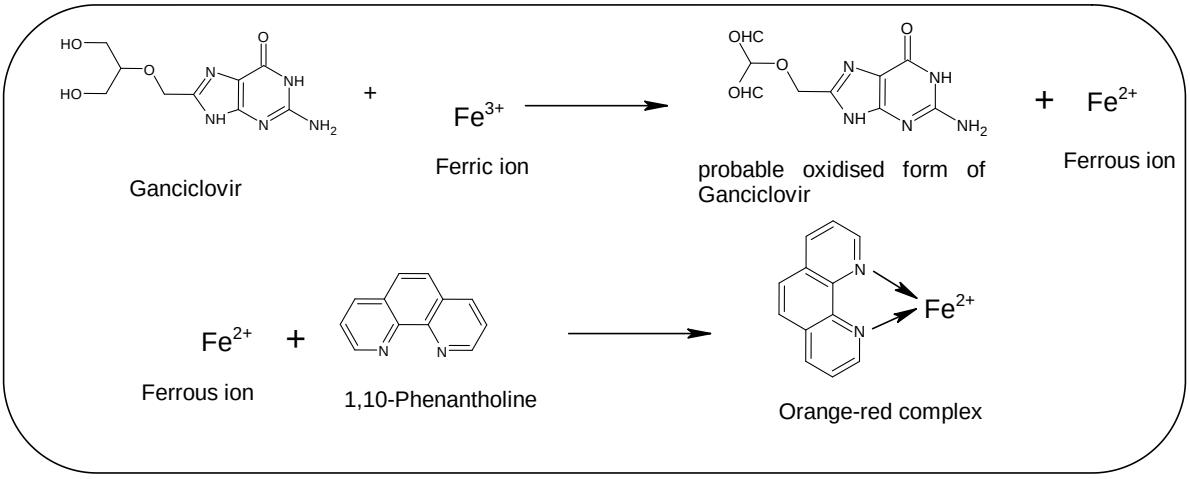

Fig. 2. Ganciclovir and Fe^{3+}/1,10-phenanthroline - probable redox-coupling reaction

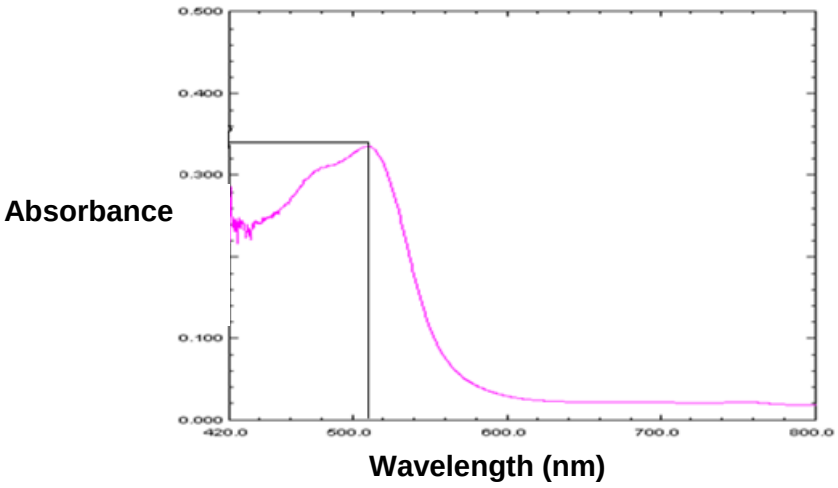

Fig. 3. UV absorption spectrum of Ganciclovir (30.0 µg/mL) in 0.1 M HCl and Fe^{3+}/1,10-phenanthroline

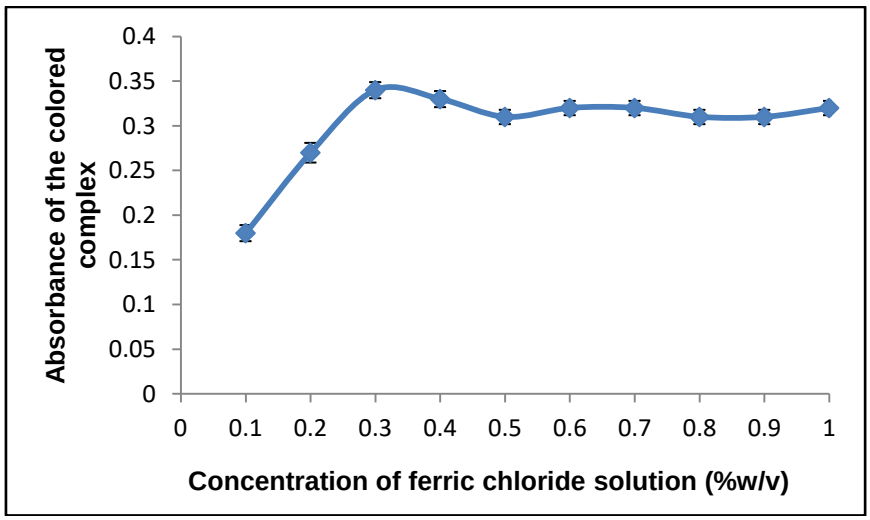

Fig. 4. Effect of ferric chloride concentration on the absorbance of colored complex

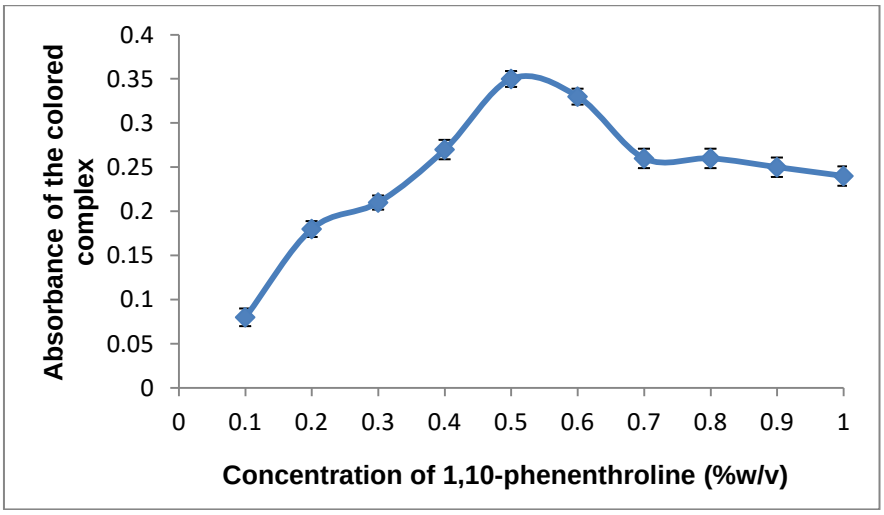

Fig. 5. Effect of 1,10-phenanthroline concentration on the absorbance of colored complex

The intensity of color development was verified at various time intervals ((5, 10, 15, 20, 25 and 30 min) to optimize the reaction. Maximum absorbance was observed at 15 min (Fig. 6). The results shown in Fig. 6 indicated that the color was stable up to 6 h under optimized conditions.

Continuous variation in the methodology was utilized to investigate the stoichiometry of the reaction. Samples containing equimolar solutions of Ganciclovir (11.75 X 10^{-5} M) and 1,10-phenanthroline were produced, while earlier mentioned reaction conditions were kept same. The drug and reagent (1,10-phenanthroline) were put together in variable mole ratios (0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2 and 0.9:0.1, respectively). The stoichiometric relation between two variables is shown in Fig. 7 and the highest absorbance was noted for a mole ratio of 0.5:0.5.

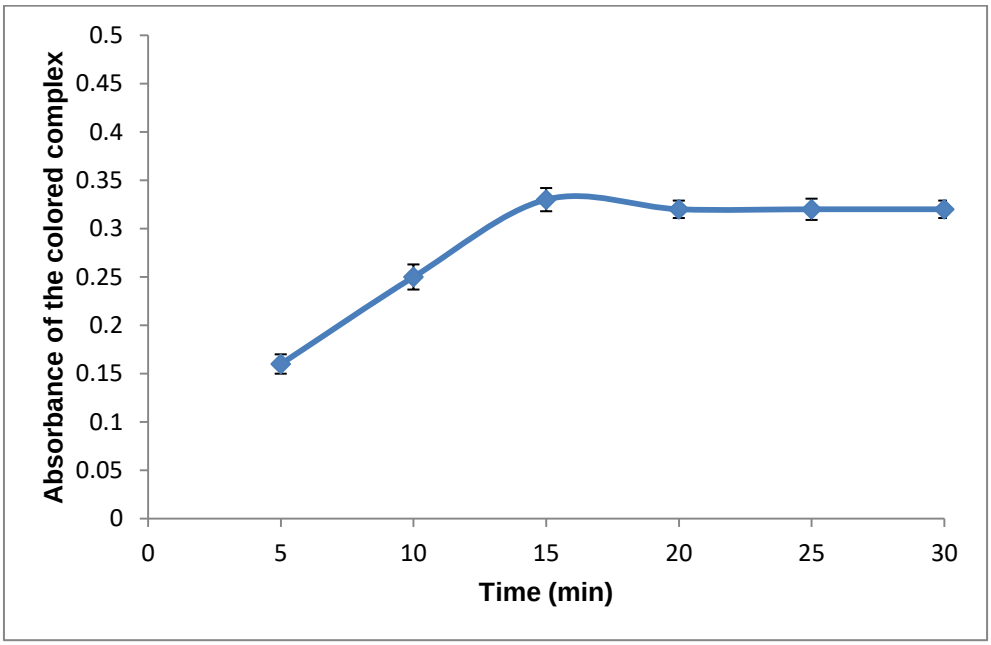

Fig. 6. Influence of reaction time on the stability of colored complex

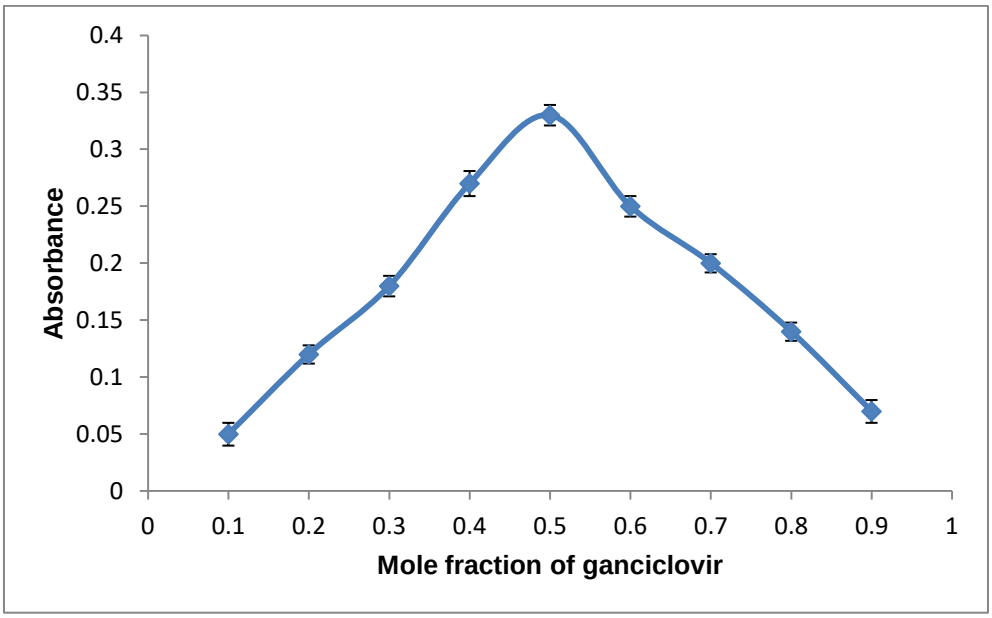

Fig. 7. Stoichiometry in the method - Job's continuous variation plot

3.2 Method Validation

The validity of the methodology was tested for the linearity, accuracy, precision, sensitivity and robustness and the results were described below.

3.2.1 Linearity

A linear relationship between the concentration of Ganciclovir and its absorbance was noticed over the concentration range of 5.0 – 30.0 µg/mL. The calibration curve was shown in Fig. 8. The linear regression equation was resulted as y = 0.011x-0.002 (Fig. 8). The correlation coefficient value (r^2) 0.999 demonstrated the linearity of the method.

3.2.2 Accuracy

The correctness in the procedure was proved by spiking the standard analyte in 80, 100 and 120% level to the fixed concentration of Ganciclovir in formulation (10.0 µg/mL). The mean of percentage recoveries and %RSD were calculated and reported in Table 2. The % recovery was found to be in the range 101.10-103.30 for Ganciclovir. The %RSD values (less than 2.0) for three levels was found to be satisfactory.

3.2.3 Precision

Three different concentrations of Ganciclovir (5.0, 15.0 and 30.0 µg/mL) were utilized to determine the repeatability (intra-day) and intermediate precision (inter-day) of the method. The data obtained was provided in Table 3. The %RSD values less than 2.0 were observed in both the studies.

3.2.4 Sensitivity and robustness

The sensitiveness of the procedure was resolute in terms of LOD and LOQ, which are calculated as 0.30 and 0.90 µg/mL, respectively (Table 4). The Sandell's sensitivity was determined and it was calculated as 9.12 X 10^{-2} µg/cm^2. The influence of minor dissimilarities in the levels of ferric chloride (0.3±0.1 w/v) and 1,10-phenanthroine (0.5±0.1% w/v) was studied to ensure robustness in the method. The outcomes disclosed that these dissimilarities did not affect the absorbance of the formed complex at a larger extent.

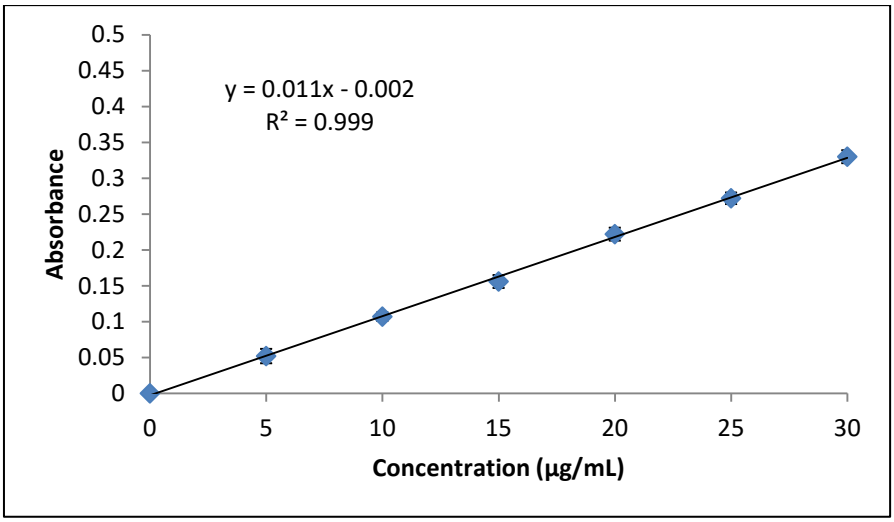

Fig. 8. Linearity plot of Ganciclovir

Table 2. Data of accuracy studies of Ganciclovir using proposed method

Drug name (brand name)	Recovery level %	Formulation (µg/mL)	Amt of drug spiked (µg/mL)	Amt of drug recovered[a] (µg/mL)	%Amt recovered	%RSD[b]
Ganciclovir (Cytovene IV)	80	10.0	8.0	8.26±0.120	103.25	1.45
	100	10.0	10.0	10.11±0.078	101.10	0.77
	120	10.0	12.0	12.4±0.117	103.30	0.94

[a]Mean of three determinations calculated with respect to the amount of drug spiked; [b]Percentage relative standard deviation

Table 3. Data of precision studies for Ganciclovir using proposed method

Conc (µg/mL)	Intra –day		Inter –day	
	Amount found[a] (AM±SD)	%RSD[b]	Amount found[a] (AM±SD)	%RSD[b]
5.0	4.90± 0.039	0.80	4.47 ±0.023	0.51
15.0	14.36±0.081	0.56	14.21±0.092	0.65
30.0	30.02±0.186	0.62	30.05±0.208	0.69

[a]Mean of three determinations with standard deviation; [b]Percentage relative standard deviation.

Table 4. Data of analytical and regression parameters obtained for Ganciclovir in the proposed method

Parameters	Values
Absorption wavelength (nm)	510.0
Beer's law range (µg/mL)	5.0 - 30.0
Limit of detection (µg/mL)	0.30
Limit of quantification (µg/mL)	0.90
Correlation coefficient (R^2)	0.999
Slope (m)	0.011
Intercept (c)	-0.002
Regression equation	y = 0.011x-0.002
Molar Absorptivity (L $mole^{-1}cm^{-1}$)	0.28 X 10^4
Sandell's sensitivity (µg/cm^2)	9.12 X 10^{-2}

Table 5. Data for assay studies of Ganciclovir using proposed method

Drug name	Formulation name	Label claim (mg)	Amount found (AM±SD)[a]	%Assay	%RSD[b]
Ganciclovir	Cytovene IV	500.0	496.0±2.731	99.2	0.55

[a]Mean value of three determinations with standard deviation; [b]Percentage relative standard deviation

3.3 Assay of Ganciclovir in Marketed Formulation

The method was applied to estimate Ganciclovir in marketed formulation (Cytovene IV). The assay results were compared with the corresponding label amount and the details were provided in Table 5. The results indicated that the excipients do not show any interference at the specified wavelength (510.0 nm). The % assay value was found to be 99.2, which lie within the acceptance criteria as mentioned in ICH guidelines.

4. CONCLUSION

The investigated redox-based colorimetric method developed for the analysis of Ganciclovir using Fe^{3+}/1,10-phenanthroline as chromogenic reagent was found to be simple and rapid. The effect of method conditions, such as concentration of ferric chloride (0.3% w/v), 1,10-phenanthroline (0.5% w/v), time for color development (15 min) and ganciclovir-1,10-phenanthroline mole ratio (0.5:0.5) were identified through optimization of the analytical method. The method was validated for parameters, such as linearity, accuracy, precision, sensitivity and robustness, in accordance with the ICH guidelines. Linearity of the method was established over the concentration range of 5.0 – 30.0 µg/mL. Analysis of Ganciclovir present in the marketed formulation utilizing the contemplated method gave satisfactory results (%assay, 99.2). The assay results also suggested that there is no mediation of formulation excipients in the determination. The LOD (0.30 µg/mL) and LOQ (0.90 µg/mL) values established in the method are considerate and indicates that this methodology can be a good alternative for the analysis of Ganciclovir at low concentration level. With the above said findings, the method can be habitually employed in quality control testing of Ganciclovir in bulk and pharmaceutical dosage forms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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