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The Use of Oxidation Reaction for the Spectrophotometric Determination of Ganciclovir in Pharmaceutical Formulations

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ABSTRACT

A simple and sensitive spectrophotometric method was developed for the determination of ganciclovir (GCV) in a bulk drug and in its pharmaceutical formulations. The method was based on the reduction of potassium permanganate by ganciclovir in alkaline medium to form a green colour product showing maximum absorbance at 610 nm. Beer's law is obeyed in the concentration range 2-100 $\mu g.ml^{-1}$ with average recovery (accuracy) 100.24% and precision (RSD) is less than 1.0%. The molar absorptivity is $4.59x10^3$ $l.mol^{-1}.cm^{-1}$ with LOD 0.21$\mu g.ml^{-1}$ and LOQ 0.72 $\mu g.ml^{-1}$. The proposed method was further applied to the determination of the drug in pharmaceutical formulations as an injection and capsule and the results are compatible with both certified values of pharmaceutical formulations and the standard addition method.

Keywords: ganciclovir; spectrophotometric; potassium permanganate; oxidation reaction.

استخدام تفاعل الأكسدة في التقدير الطيفي للغانسيكلوفير في المستحضرات الصيدلانية

الملخص

تم تطوير طريقة طيفية بسيطة وسريعة لتقدير المركب الدوائي غانسيكلوفير بهيئته النقية وفي مستحضراته الصيدلانية. اعتمدت الطريقة على اختزال برمنكنات البوتاسيوم بالمركب الدوائي لتكوين ناتج اخضر الذي يقاس أقصى امتصاص له عند 610 نانوميتر. وكانت حدود تطبيق قانون بير من 2 إلى 100 مايكروغرام.مللتر$^{-1}$ و بدقة وتوافق مرضيين. إذ بلغ معدل نسبة الاسترجاع 100.24% والانحراف القياسي النسبي اقل من 1.0%. بلغت الامتصاصية المولارية 4.59×10^3 لتر . مول$^{-1}$. سم$^{-1}$ بحدود كشف 0.21 مايكروغرام.مللتر$^{-1}$ وتقدير كمي 0.72 مايكروغرام.مللتر$^{-1}$. طبقت الطريقة بنجاح في تقدير الغانسيكلوفير في المستحضرات الصيدلانية (الحقن والكبسول) ووجد أن نتائج الطريقة متفقة مع محتوى المستحضر الصيدلاني وكذلك مع طريقة الإضافة القياسية.

INTRODUCTION

Ganciclovir (GCV) or 9-(1,3-dihydroxy-2-propoxymethyl) guanine (Fig. 1), is a cyclic nucleoside analogue of 2-deoxyguanosine that inhibits replication of herpes viruses. It is used for the prevention of cytomegalovirus (CMV) disease in organ or bone marrow transplant recipients and in HIV-infected individuals who are at risk of developing CMV disease. GCV is a white crystalline powder with a molecular formula of $C_9H_{13}N_5O_4$ and a molecular weight 255.23 g/mol (Sweetman, 2005 ; Bertam , 2007).

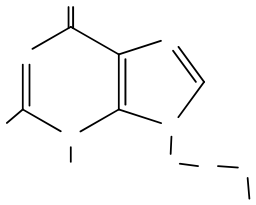


Fig. 1 : The chemical structure of Ganciclovir

Few analytical methods have been reported for the estimation of GCV in biological fluids or pharmaceutical formulations such as High – performance liquid chromatographic (HPLC) analysis of GCV in plasma samples using a mobile phase 0.02 M KH_2PO_4 with UV detection at 254 nm (Boulieu *et al*., 1991). Or capillary electrophoresis method which was used for GCV determination in human plasma with UV detection at 254 nm (Saleh and Hempel, 2006).

The determination of GCV in human serum and pharmaceutical dosage forms was also investigated using differential pulse and square wave voltammetry (Uslu *et al*., 2005). Finally the spectrophotometric methods have been developed for the determination of GCV in bulk drug and its pharmaceutical formulations such as the reaction of GCV with p-dimethylamino cinnamaldehyde (Sarsambi *et al*., 2010), a charge transfer reaction of GCV (n- electron donor) with several σ and π acceptors (Gouda, 2009) and first order derivative spectroscopy (Sarsambi *et al*., 2010). GCV also was estimated at 253 nm in 0.1 N HCl and at 266 nm in 0.1 N NaOH (Sarsambi *et al*., 2010). Eight direct spectrophotometric methods for determination of ganciclovir has been developed. These methods were based on the oxidation of the drug by different inorganic oxidants in acidic medium (Gouda and Amin, 2011).

The potassium permanganate has been used in pharmaceutical analysis in the determination of tetracycline, for example (Ahmidaa *et al*., 2009), pantoprazole (Basavaiah *et al*., 2009), labetalol (Rahman *et al*. , 2011), furosemide (Tharpa *et al*., 2009), olanzapine (Rajendraprasad and Basavaiah, 2009), amlodipine besylate (Shama *et al*., 2010), ciprofloxacin and lomefloxacin (Darwish *et al*., 2010), enalapril maleate (Vinay *et al*., 2010), piroxicam and tenoxicam (Amin *et al*., 2010), pipazethate and dextromethorphan (Gouda *et al*., 2008), hyoscine butylbromide (Gouda, 2010) and metronidazole benzoate (Farhadi and Bahar, 2007).

The aim of the present work is to develop a simple, sensitive and cost-effective spectrophotometric method for the determination of GCV in pharmaceutical formulations. The method makes use the potassium permanganate as an oxidimetric reagent, and has been

demonstrated to be superior to the existing spectrophotometric method in terms of sensitivity, colour stability, working conditions, accuracy and precision.

EXPERIMENTAL

Apparatus

A shimadzu UV – 1650 a digital double beam spectrophotometer with 1- cm glass cells was used for all spectral and absorbance measurements.

Reagents

GCV was provided from (European Directorate for the Quality of Medecines and HealthCare) and potassium permanganate from (Merck, Darmstadt, Germany).

Standard GCV (1000 $\mu g.ml^{-1}$) solution

A stock solution of GCV was prepared by dissolving 0.1000 g in sufficient quantity of distilled water and the volume was made up to 100 ml with distilled water. Further dilution was made with distilled water to get the concentration of 100 $\mu g.ml^{-1}$.

Potassium permanganate ($5x10^{-3}$ M) aqueous solution

This solution was prepared by dissolving 0.079 g in water and diluting to 100 ml in a calibrated flask, and standardized against sodium oxalate in acidic medium (Bright, 1961).

Sodium hydroxide solution (1 M)

This solution was prepared by dissolving 4 g of sodium hydroxide from (Merck) in 100 ml of distilled water.

Assay procedure for dosage forms

1- Injection:

A vial of cymevene (IV) from Roche contains 500 mg ganciclovir. The content of two vials were mixed and an amount equivalent to 500 mg of the powdered component was weighed and dissolved in distilled water and filtered then completed to the mark in a 100 ml volumetric flask with distilled water, from the above solution 20 ml was pipette out into a 100 ml volumetric flask and the volume was made up to the mark with distilled water. Further dilution was made with distilled water to get the concentration of 100 $\mu g.ml^{-1}$. The solution was proceeded as described under procedure for calibration.

2- Capsule:

An accurately weighed quantity of the mixed contents of 10 capsules (Lovir from Oubari Pharma – Aleppo – Syria), an amount equivalent to 250 mg of the drug was dissolved in sufficient quantity of distilled water and filtered then the volume was made up to 100 ml with distilled water. From the above solution 40 ml was pipette out into a 100 ml volumetric flask

and the volume was made up to the mark with distilled water. Further dilution was made with distilled water to get the concentration of 100 μg.ml^{-1}. The solution was proceeded as described under procedure for calibration.

RESULTS AND DISCUSSION

The absorption spectrum of $KMnO_4$ in basic medium shows a maximum absorption band at 530 nm (Fig. 2). The addition of aqueous solution of GCV to $KMnO_4$ solution in basic medium produce a green coloured product which shows a new characteristic absorption band at 610 nm.

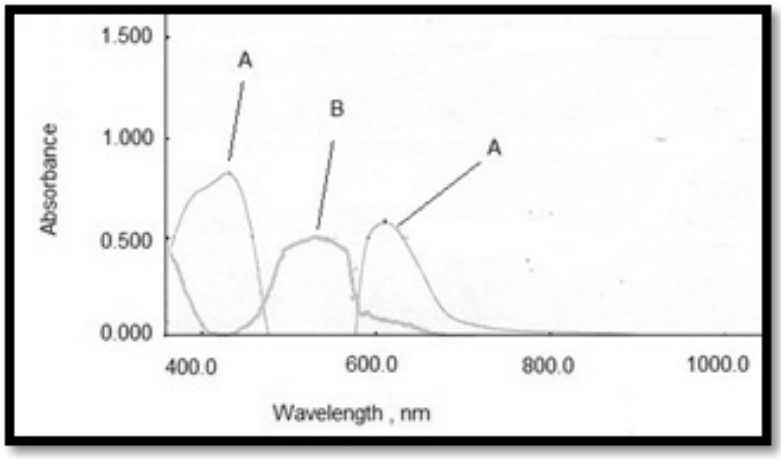


Fig. 2: Absorption spectra of (A) GCV-$KMnO_4$ product and (B) of 5.0×10^{-3} M $KMnO_4$ solution in alkaline medium.

Optimization of conditions

For the subsequent experiments, 300 μg of GCV is taken and the final volumes were 10 ml.

Effect of $KMnO_4$

The effect of different $KMnO_4$ amounts on the absorbance of solution containing 30 μg. ml^{-1} GCV were studied and reached maximum on using 1.5 ml of $5x10^{-3}$ M $KMnO_4$. Therefore, this volume was used all subsequent work (Table 1).

Table 1: Effect of $KMnO_4$.

ml of $KMnO_4$ solution $5x10^{-3}$ M	0.5	1.0	1.5	2.0	2.5
Absorbance	0.278	0.465	0.586	0.576	0.565

Effect of base

Various bases such as KOH, NaOH, Na_2CO_3 and NH_4OH were examined in order to obtain high sensitivity and selectivity for the determination of GCV, It was found that the using of NaOH gave a maximum colour intensity. Therefore, it was used in this study (Table 2).

Table 2 : Effect of base.

Type of alkaline medium (1 M)	**NaOH**	**KOH**	**Na_2CO_3**	**NH_4OH**
Absorbance	0.584	0.576	0.210	0.101

Effect of NaOH

The influence of NaOH amounts on the absorbance of the reaction product were also studied. A maximum colour intensity was shown on using 1 ml of 1M NaOH. Therefore, this amount was used in this study (Table 3).

Table 3: Effect of NaOH.

ml of sodium hydroxide (1 M)	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**
Absorbance	0.254	0.301	0.385	0.431	0.585	0.576	0.498

Effect of time and temperature:

The effect of reaction time was studied by following the colour intensity at room temperature and in thermostatically controlled water – bath adjusted at 50 and 60 C°. This study showed that the coloured dye was developed after 10 minutes and the absorbance remained stable for at least 6 hours. All conditions studied were optimized at room temperature (25±1 C°) which give the best color intensity (Table 4).

Table 4 : Effect of temperature and reaction time.

Temp. (C°)	**Absorbance**							
	Time (min)							
	5	10	20	40	60	2h	4h	6h
R.T	0.418	0.586	0.585	0.586	0.584	0.584	0.585	0.584
50	0.420	0.589	0.581	0.574	0.562	0.551	0.542	0.442
60	Turbid	-	-	-	-	-	-	-

Effect of surfactant:

The effect of different types of surfactants was studied, but none of them improve the absorption intensity therefore they were excluded from this study (Table 5).

Table 5: Effect of surfactant.

Surfactant	Absorbance/ml surfactant			
	0.5	1	2	3
Cetyltrimethylammonium bromide (0.1%)	0.576	0.565	0.543	0.510
Sodium dodecyl sulphate (0.1%)	0.564	0.550	0.504	0.491
Triton x-100 (1%)	0.543	0.532	0.526	0.479
Without surfactant	0.586			

Effect of sequence of additions

Drug-base-oxidant is the optimum sequence of addition; other sequences gave lower absorbance values under the same experimental conditions.

Reaction components	Absorbance
Drug-base-oxidant	0.585
Drug-oxidant-base	0.274
Oxidant-base-drug	0.387

Procedure for calibration

Transfer aliquot volumes of GCV standard solution covering the working concentration range from 2 to 100 µg**.** ml^{-1} into 10 ml volumetric flasks, add 1 ml of 1 M NaOH followed by 1.5 ml of $5x10^{-3}$ M potassium permanganate and shake well, then make up to the mark with distilled water. Allow the reaction mixture to stand for 10 min. Measure the absorbance of the resulting solution at 610 nm against a reagent blank prepared simultaneously.

Analytical data

Beer's law plot was obeyed in the concentration range (2-100) $\mu g.ml^{-1}$ for GCV (Fig. 3) with a correlation coefficient, molar absorptivity and regression equation were given in (Table 6). The recovery and the relative standard deviation (RSD) were determined at three different concentrations. The results shown in (Table 7) indicate very good accuracy (average recovery %) 100.24% while the RSD is < 1.0%.

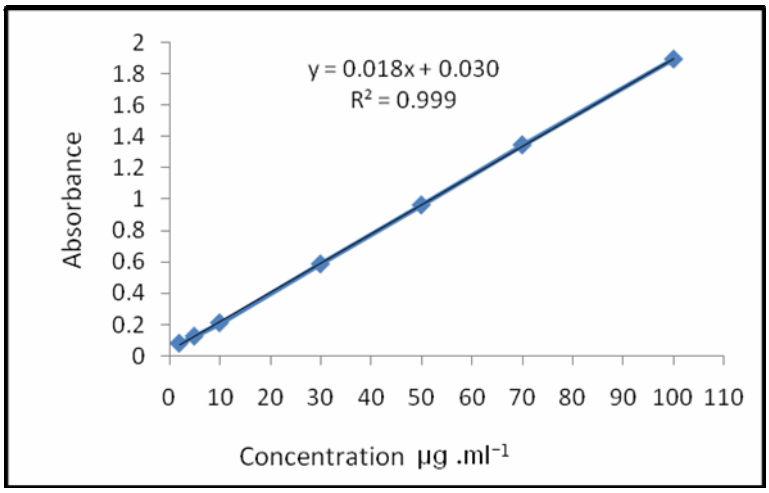


Fig. 3: Calibration graph of Ganciclovir

Table 6: Quantitative parameters.

Parameter	Values
λ_{max} (nm)	610
Beer's law limit (µg.ml^{-1})	2-100
Molar absorptivity (l.mol^{-1}.cm^{-1})	4.59x10^{3}
Regression equation(Y)*	
Slope (a)	0.018
Intercept(b)	0.030
Correlation coefficient	0.9990
LOD (µg.ml^{-1})	0.21
LOQ (µg.ml^{-1})	0.72

*Y=ax+b , where x is the concentration of GCV in µg.ml^{-1} and Y is the absorbance

Table 7 : Accuracy and precision of the method.

Taken (µg . ml^{-1})	Found (µg . ml^{-1})	Recovery* (%)	RSD* (%)
10	10.04	100.48	±0.71
30	30.05	100.17	±0.25
70	70.04	100.07	±0.10

- Average of six determinations

Interferences

In pharmaceutical analysis methods, it is important to test the selectivity of the methods towards excipients and additives added to the pharmaceutical preparations of GCV. It is clear from the results obtained from (Table 8) that the commonly encountered excipients did not interfere with proposed method indicating a high selectivity for determining GCV in its dosage forms.

Table 8: Effect of interferences.

Excipient	Recovery % µg of excipient added			
	500	1000	2000	3000
Starch	100.96	101.10	103.22	104.76
Magnesium stearate	102.35	102.55	103.43	102.99
Croscarmellose sodium	100.98	101.87	102.24	103.32
Microcrystalline cellulose	101.77	102.65	101.58	104.58

Nature of product and reaction mechanism

The stochiometry of the reaction between GCV and $KMnO_4$ was investigated using Job's method (Delevie, 1997). The results obtained show that the product was formed in the ratio 1:2 (GCV : $KMnO_4$) (Fig.4).

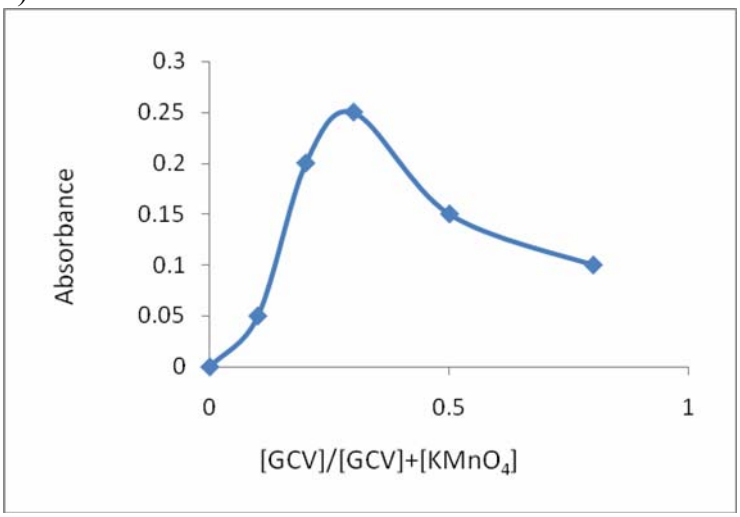
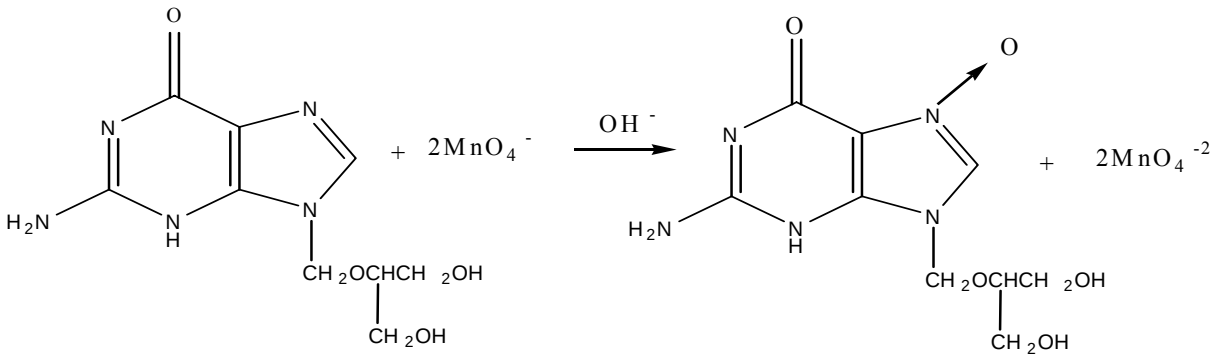


Fig. 4: Job's method graph for GCV-KMnO4 product

Therefore, the formation of the product may be occur as follows :



The stability constant of the product in basic medium , under the conditions of experimental procedure was calculated, and found to be $1.6x10^9$ $l^2.mol^{-2}$.

Application

The proposed method was applied to the determination of GCV in pharmaceutical formulations. Good recovery was obtained (Table 9) and the results compared with the standard addition method (Fig. 5, Fig.6 and Table 10).

Table 9 : Assay and recovery of GCV in pharmaceutical dosage forms.

Pharmaceutical dosage forms	Certified value	Amount of GCV added ($\mu g . ml^{-1}$)	Drug content found (mg)	Recovery*(%)
Cymevene (Injection)	500mg/vial	10	492.25	98.45
		30	497.35	99.47
Lovir (Capsule)	250mg/capsule	10	244.72	97.89
		30	248.90	99.56

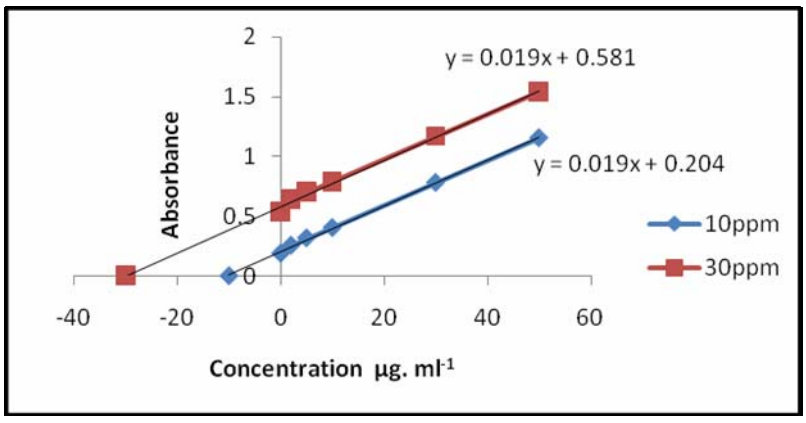


Fig. 5 : Standard addition graph of GCV in pharmaceutical formulation (injection).

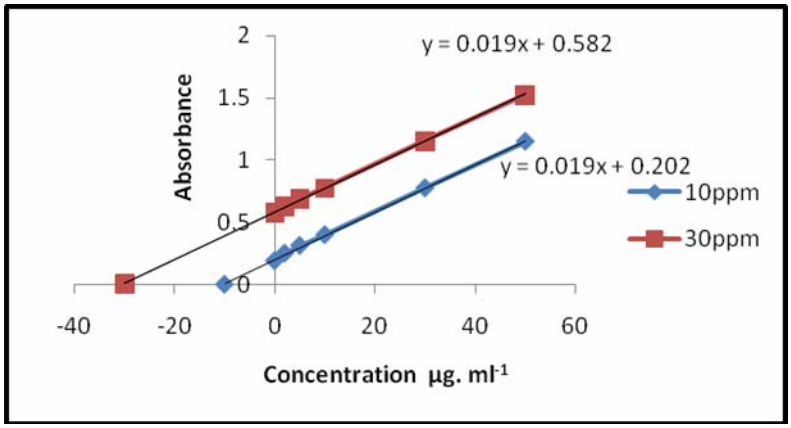


Fig. 6 : Standard addition graph of GCV in pharmaceutical formulation (capsule).

Table 10 : Comparison of the proposed method with standard addition method.

Pharmaceutical dosage forms	Certified value	Amount of GCV added (µg . ml^{-1})	Recovery*(%)	
			Present method	Standard addition method
Cymevene (Injection)	500mg/vial	10	98.45	107.63
		30	99.47	101.90
Lovir (Capsule)	250mg/capsule	10	97.89	106.30
		30	99.56	102.10

*Average of three determinations.

The experimental t-test for GCV injection found to be 2.04, while the tabulated value is 4.303 at confidence level 95% and for two degree of freedom, and the experimental t-test for GCV capsule found to be 2.42, while the tabulated value is 4.303 at confidence level 95% and for two degree of freedom, which indicate that this method is reliable for application.

Comparison of methods

The results obtained by application of the present method and literature method (Sarsambi *et al*., 2010) to the determination of GCV in pharmaceutical preparations were given in (Table 11).

Table 11: Comparison of methods.

Analytical parameter	Present method	Literature method
λ_{max} (nm)	610	524
Temp (°C)	R.T	40
Linear range (µg. ml^{-1})	2-100	10-50
Molar absorptivity(l. mol^{-1}. cm^{-1})	$4.59x10^3$	1.175×10^3
Limit of Detection (LOD/ µg. ml^{-1})	0.21	0.425
Limit of Quantification (LOQ/ µg. ml^{-1})	0.72	4.60
Type of reaction	Oxidation	Condensation
Composition of the dye	1:2	1:1
Analytical application	Injection and capsules	Capsules

It is evident from table 11 that the present method is more sensitive than the most recently –published method on GCV determination.

CONCLUSION

$KMnO_4$ is a suitable reagent for the determination of GCV in pure form or in its dosage forms. The suggested method is simple and does not require solvent extraction.

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