Spectrophotometric Estimation of Ganciclovir in Pharmaceutical Formulations using Alizarin Sulfonic Acid Sodium Salt Reagent

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الملخص

تم تطوير طريقة طيفية بسيطة وسريعة لتقدير المركب الدوائي غانسيكلوفير بهيئته النقية وفي مستحضراته الصيدلانية. اعتمدت الطريقة على تفاعل تكوين مزدوج ايوني للمركب الدوائي مع كاشف ملح الصوديوم اليزارين حامض السلفونيك في المحلول المائي لتكوين ناتج وردي محمر يقاس أقصى امتصاص له عند 525 نانوميتر. وكانت حدود تطبيق قانون بير من 2 إلى 100 مايكروغرام.مللتر$^{-1}$ و بدقة وتوافق مرضيين. إذ بلغ معدل نسبة الاسترجاع 99.80% والانحراف القياسي النسبي اقل من 1.0%. بلغت الامتصاصية المولارية $4.59x10^3$لتر.مول$^{-1}$.سم$^{-1}$ بحدود كشف 0.17 مايكروغرام.مللتر$^{-1}$ وتقدير كمي 0.56 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.

ABSTRACT

A simple and rapid spectrophotometric method has been developed for the determination of antiviral drug (Ganciclovir) in a bulk sample and in its dosage forms. The method depends on the ion-pair formation reaction of the drug with alizarin sulfonic acid sodium salt reagent in aqueous solution to form a pinkish - red color product showing maximum absorbance at 525 nm. Beer's law is obeyed in the concentration range 2 – 100 µg.ml^{-1} with average recovery (accuracy) 99.80% and precision (RSD) is less than 1.0%. The molar absorptivity is $4.59x10^3$ l.mol^{-1}.cm^{-1} with LOD 0.17 µg.ml^{-1} and LOQ 0.56 µg.ml^{-1}. The method is successfully employed for the determination of Ganciclovir 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; alizarin sulfonic acid sodium salt**.**

Introduction

Ganciclovir (GCV) or 9-(1,3-dihydroxy-2-propoxymethyl) guanine, Fig.(1) is an acyclic 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 [1, 2].

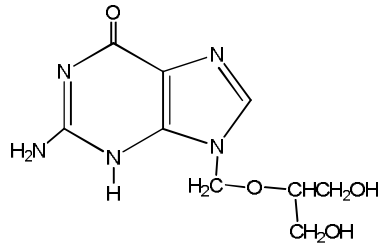


Fig. 1: The chemical structure of Ganciclovir

Few analytical methods have been reported for the estimation of GCV in biological fluids or pharmaceutical formulations. 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 was investigated [3].

Also capillary electrophoresis method was used for GCV determination in human plasma with UV detection at 254 nm [4].

The determination of GCV in human serum and pharmaceutical dosage forms was investigated using differential pulse and square wave voltammetry [5].

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 [6], a charge transfer reaction of GCV (n- electron donor) with several σ and π acceptors [7] and first order derivative spectroscopy [8]. GCV also was estimated at 253 nm in 0.1 N HCl and at 266 nm in 0.1 N NaOH [9].

Alizarin sulfonic acid sodium salt has been frequently utilized as an analytical reagent in pharmaceutical analysis. It has been used for the colorimetric determination of ceterizine hydrochloride at 440 nm[10], clotrimazole and ketoconazole at 425 nm[11], piroxicam and tenoxicam[12], nefopam, mebevrine and phenylpropanol amine hydrochloride [13].

Spectrophotometric analysis is considered more convenient alternative technique to determine GCV because of its simplicity and its high sensitivity. Therefore the objective of this work was to develop a

simple spectrophotometric method to determine the drug in the pure form and its pharmaceutical formulations.

Experimental

Apparatus

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

Reagents

GCV from (European Directorate for the Quality of Medecines & HealthCare), alizarin sulfonic acid sodium salt hydrate ($C_{14}H_7NaO_7S.H_2O$) analytical reagent grade from Merck (Germany) were used.

Standard GCV (1000 µg.ml^{-1}) solution

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

Alizarin sulfonic acid sodium salt hydrate (1x 10^{-3} M) solution

This solution was prepared by dissolving 0.0171 g alizarin sulfonic acid sodium salt hydrate in distilled water and then the volume was made up to a 50 ml with distilled water in a 50 ml volumetric flask.

Procedure for calibration

To a series of 10 ml volumetric flasks, transfer increasing volumes of GCV working standard solution to cover the range (2 – 100) µg.ml^{-1} in final dilution. Add 2 ml of 1.00 x 10^{-3} mol.L $^{-1}$ alizarin sulfonic acid sodium salt hydrate. Dilute the solution to the mark with distilled water. The absorbance was measured at 525 nm after 10 minutes at room temperature against the blank solution which was prepared in a similar way but without the addition of GCV.

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 of the powder equivalent to 500 mg of the component was weighed and dissolved in distilled water and filtered then completed to the mark in a 100 ml volumetric flask, 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 µg.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 GCV was measured in the range 400-800 nm against the blank solution. GCV was reacted with alizarin sulfonic acid sodium salt hydrate to produce an pinkish- red colored ion – pair complex with maximum absorption at 525 nm in contrast to the reagent blank which shows maximum absorption at 420 nm Fig.(2).

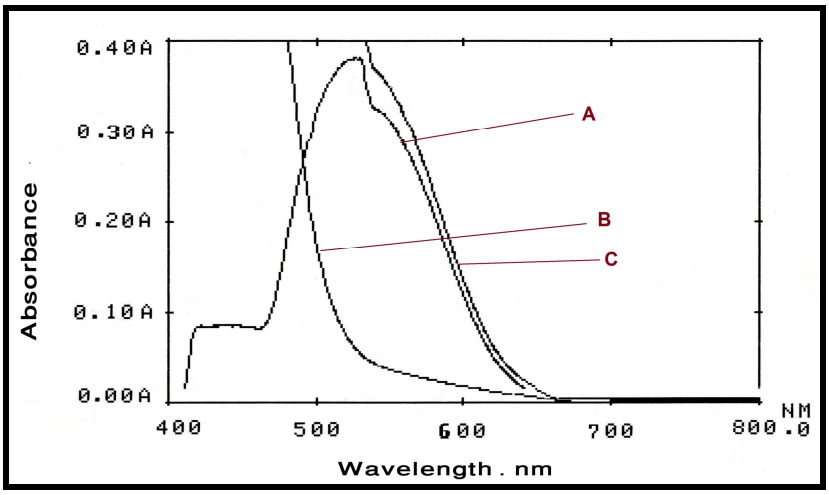


Fig. 2: Absorption spectra of 20 µg. ml^{-1} of GCV treated according to the procedure and measured against blank (A), blank against distilled water (B), GCV treated according to the procedure and measured against distilled water (C).

Optimization of conditions

For the subsequent experiments, 200 µg of GCV is taken and the final volumes are 10 ml.

Effect of pH:

Since alizarin sulfonic acid sodium salt hydrate behaves as an acid – base indicator so neither acid nor base were used in the reaction medium [14].

Effect of the reagent concentration:

The effect of the reagent was investigated by taking various amounts (0.5-3) ml of the reagent which was added to an aliquot of solution containing 200 µg.10 ml^{-1} of GCV and following the general

procedure. The better absorption was observed with the addition of 2 ml of the reagent (Table 1).

Table 1: Effect of alizarin sulfonic acid sodium salt concentration

ml of alizarin sulfonic acid sodium salt hydrate ($1x10^{-3}$ M)	0.5	1	1.5	2.0	2.5	3.0
Absorbance	0.099	0.158	0.331	0.385	0.298	0.178

Effect of time and temperature:

The reaction time was determined by following the color intensity at room temperature and in thermostatically controlled water – bath adjusted at 50 and 60 C°. The experiment showed that the colored dye developed immediately 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 2).

Table 2: Effect of temperature and reaction time

Temp (C°)	Absorbance							
	Time (min)							
	5	10	20	40	60	2h	4h	6h
R.T	0.385	0.384	0.385	0.385	0.385	0.385	0.385	0.385
50	0.382	0.381	0.377	0.372	0.368	0.350	0.332	0.319
60	0.379	0.378	0.369	0.367	0.361	0.348	0.323	0.309

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 3).

Table 3: Effect of surfactant

Surfactant	Absorbance/ml surfactant			
	0.5	1	2	3
Cetyltrimethylammonium bromide (0.1%)	0.380	0.378	0.377	0.375
Sodium dodecyl sulphate (0.1%)	0.381	0.379	0.380	0.378
Triton x-100 (1%)	0.382	0.380	0.378	0.377
Without surfactant	0.385			

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, regression equation were given in Table 4. In order to determine the accuracy and precision of the proposed method the recoveries (R %) and relative standard deviation (RSD %) for three different concentrations of drug were also calculated and recorded in Table 5.

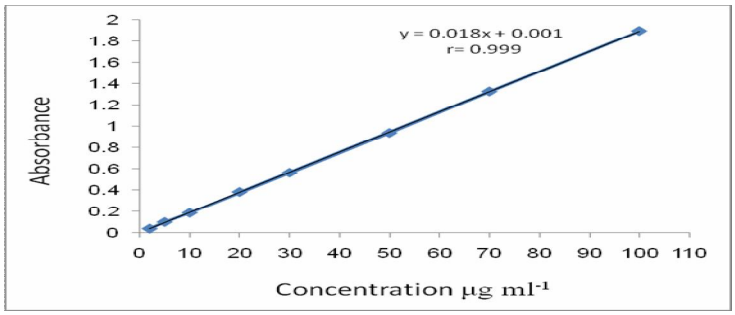


Fig. 3: Calibration curve of Ganciclovir

Table 4: Quantitative parameters for the complexation of GCV with alizarin sulfonic acid sodium salt hydrate.

Parameter	Values
λ_{max} (nm)	525
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)	0.001
Correlation coefficient (r)	0.999
LOD (µg.ml^{-1})	0.17
LOQ (µg.ml^{-1})	0.56

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

Table 5: Accuracy and precision of the method.

Taken (µg. ml^{-1})	Found (µg. ml^{-1})	Recovery* (%)	RSD* (%)
20	19.94	99.70	0.50
50	49.91	99.82	0.15
70	69.92	99.88	0.11

* Average of six determinations

Interferences

In order to assess the possible analytical application of this spectrophotometric method to drug quality control, the interfering effect of some common excipients at various levels on the determination of GCV was examined and the results were given in (Table 6).

Table 6: Effect of interferences

Excipient	Recovery % µg of excipient added			
	500	1000	2000	3000
Starch	**101.51**	**102.87**	**103.65**	**104.91**
Magnesium stearate	**98.70**	**103.93**	**104.87**	**103.23**
Croscarmellose sodium	**101.12**	**99.23**	**98.54**	**102.56**
Microcrystalline cellulose	**98.56**	**99.76**	**101.21**	**103.32**

From the results in Table (6), it can be observed that none of the excipients can introduce significant interference.

Nature of product and reaction mechanism

The stiochiometry of the reaction between the drug and the reagent was investigated using continuous variation method[15], the results obtained show that 1:1 drug to reagent was formed Fig. (4).

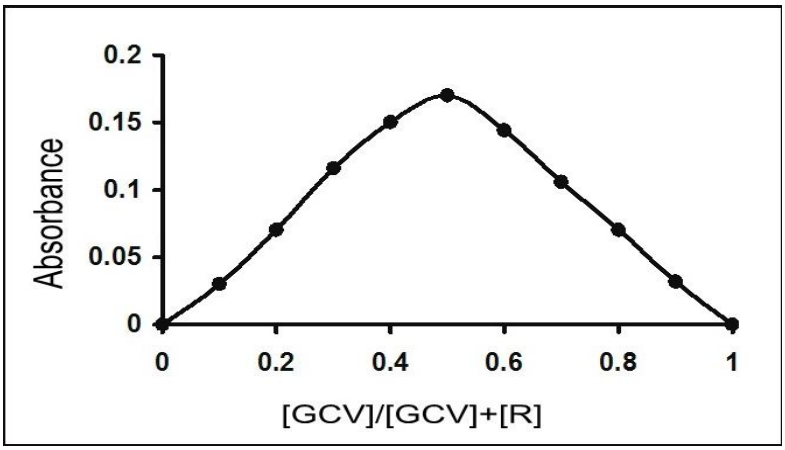
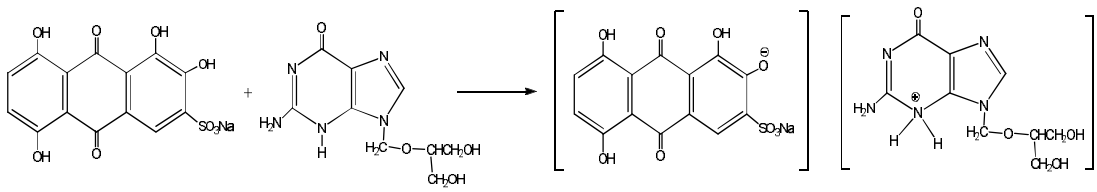


Fig. 4: Continuous variation plot of GCV with alizarin sulfonic acid sodium salt (R) reagent

Therefore, the formation of the product may be occur as follows [16-18].



The stability constant of the ion-pair complex in aqueous solution, under the conditions of experimental procedure was calculated, and found to be 1.78×10^4 l.mol^{-1}.

Application

The proposed method was applied to the determination of GCV in pharmaceutical formulations. Good recovery was obtained and the results compared with the standard addition method (Fig. 5, Fig.6 and Table 7).

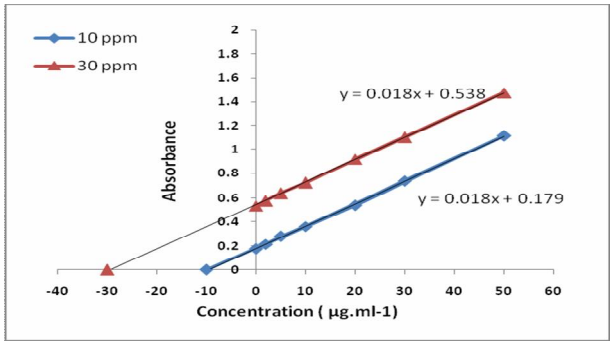


Fig.5: Standard addition graph of GCV in pharmaceutical formulation (injection)

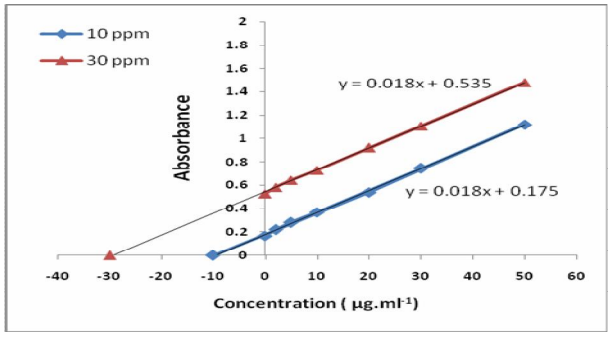


Fig.6: Standard addition graph of GCV in pharmaceutical formulation (capsule)

Table 7: Assay and recovery of GCV in pharmaceutical dosage forms.

Pharmaceutical dosage form	Certified value	Amount of GCV added ($\mu g.ml^{-1}$)	Drug content found (mg)		Recovery*(%)	
			Present method	Standard addition method	Present method	Standard addition method
Cymevene (Injection)	500mg/vial	10	498.10	494.45	99.62	98.89
		30	498.55	497.20	99.71	99.44
Lovir (Capsule)	250mg/capsule	10	248.00	241.70	99.20	96.68
		30	247.05	247.22	98.82	98.88

*Average of three determinations

Comparison of methods

The results obtained by application of the present method and literature method[6] to the determination of GCV in pharmaceutical preparations were given in (Table 8).

Table 8: Comparison of methods

Analytical parameter	Present method	Literature method[6]
λ_{max} (nm)	525	524
Temp (°C)	R.T	40
Linear range ($\mu g.\ ml^{-1}$)	2-100	10-50
Molar absorptivity ($l.\ mol^{-1}.\ cm^{-1}$)	$4.59x10^3$	1.175×10^3
Limit of Detection (LOD/$\mu g.\ ml^{-1}$)	0.17	0.425

Limit of Quantification (LOQ/µg. ml^{-1})	**0.56**	**4.60**
Type of reaction	**Ion –pair complex**	**Condensation**
Composition of the dye	**1:1**	**1:1**
Analytical application	**Injection and capsules**	**Capsules**

It is evident from Table 8 that the present method is more sensitive than the most recently –published method on GCV determination.

Conclusion

Alizarin sulfonic acid sodium salt hydrate 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 neither buffer solution nor solvent extraction.

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