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Simple spectrophotometric determination of acyclovir in bulk drug and formulations

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Abstract

A simple and cost effective spectrophotometric method is described for the determination of acyclovir in bulk drug and in formulations. The method is based on the formation of blue coloured chromogen when the drug reacts with Folin–Ciocalteu (F–C) reagent in alkaline medium. The coloured species has an absorption maximum at 760 nm and obeys Beer's law in the concentration range 50–450 μg ml^{-1}. The absorbance was found to increase linearly with increasing concentration of acyclovir, which is corroborated by the calculated correlation coefficient value of 0.9998 ($n = 9$). The apparent molar absorptivity and Sandell sensitivity were 1.65×10^2 l mol^{-1} cm^{-1} and 1.36 μg cm^{-2}, respectively. The slope and intercept of the equation of the regression line are 6.87×10^{-4} and 8.33×10^{-3}, respectively. The limit of detection was 5.68 μg ml^{-1} and the limit of quantification was 18.95 μg ml^{-1}. The proposed method was successfully applied to the determination of acyclovir in pharmaceutical formulations. The reliability of the assay method was established by parallel determination by standard-addition method, and by recovery studies. The results demonstrated and the procedure is at least as accurate, precise and reproducible (RSD < 2%) as the official method, while being simple and less time consuming. A statistical analysis indicated that there was no significant difference between the results obtained by the proposed procedure and those of the official method. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Acyclovir; Spectrophotometry; Determination; F–C reagent; Formulations

1. Introduction

Acyclovir, (9,2-hydroxyethoxy) methyl guanine, whose structure is given in Fig. 1, is an antiviral drug used extensively in the treatment of skin infections caused by herpes simplex virus [1]. It is official in European Pharmacopoeia [2], British Pharmacopoeia [3] and United States Pharmacopoeia [4]. The therapeutic importance of this drug has prompted the development of many methods for its assay. The most extensively used technique for the quantitation of acyclovir is HPLC but, most of the procedures using this technique are devoted to body fluids like plasma [5–10], serum [11–13], serum and urine [14], and plasma and urine [15]. Even such techniques as radio immuno assay [16,17], high-performance capillary electrophoresis [18], liquid chromatography [19] and micellar liquid chromatography [20] are also confined to biological fluids including plasma and urine [16], plasma [17,19], urine [18], and serum and plasma [20]. Three methods involving HPLC technique have been applied for the determination of acyclovir in pharmaceutical formulations [21–23], but these procedures lack sensitivity with the concentration range being 0.1–1.0 mg ml^{-1} [18] and 50–200 μg ml^{-1} [23] besides being tedious and difficult to perform.

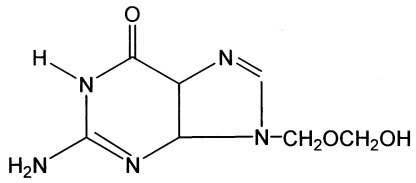

Fig. 1. Structure of acyclovir.

Methods based on derivative [24] and differential [25] spectrophotometry have also been reported for the assay of acyclovir in dosage forms. To the best of our knowledge, there is no work in the literature reported

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about the visible spectrophotometric method for the analysis of acyclovir in either biological fluids or pharmaceutical formulations. The purpose of this investigation was to develop a simple and sensitive visible spectrophotometric method for the quantitation of acyclovir in pure drug and in pharmaceutical formulations. The method uses the well known reduction reaction involving Folin–Ciocalteu (F–C) reagent and acyclovir resulting in the formation a blue chromogen that could be measured at 760 nm.

2. Experimental

2.1. Apparatus

A Systronic model 106 digital spectrophotometer with 1-cm matched glass cells was used for absorbance measurements.

2.2. Reagents and solutions

All chemicals used were of analytical reagent grade and double distilled water was used throughout. F–C reagent (2 N) supplied by S.d. Fine chem. India, Ltd., was used directly. Sodium carbonate solution (20%) was prepared in water and filtered.

2.3. Standard drug solution

Pharmaceutical grade acyclovir was kindly gifted by Cipla India Ltd., Mumbai, and was used as received. A stock solution containing 1000 μg ml^{-1} of acyclovir was prepared by dissolving 100 mg of the sample in water and diluting to 100 ml in standard flask.

2.4. Dosage forms

The following commercial acyclovir formulations were subjected to analysis by the proposed procedure. Acyvir DT (200 mg drug, 50 mg starch, 50 mg talc, 20 mg magnesium stearate and 20 mg sodium alginate); Acyvir DT (400 mg drug, 40 mg starch, 20 mg talc, 30 mg magnesium stearate and 20 mg sodium alginate); Acyvir DT (800 mg drug, 50 mg starch, 50 mg talc, 30 mg magnesium stearate and 30 mg sodium alginate).

Ocvir, 200 mg (200 mg drug, 40 mg starch, 35 mg talc, 30 mg magnesium stearate and 20 mg sodium alginate); Ocvir 400 mg (contains 400 mg of drug, 50 mg starch, 40 mg talc, 30 mg magnesium stearate and 10 mg sodium alginate); Ocvir (800 mg drug, 50 mg starch, 50 mg talc, 30 mg magnesium stearate and 25 mg sodium alginate). Ocvir suspension (400 mg drug in 5 ml in a flavoured syrup base).

2.5. Procedure

2.5.1. General procedures

Different aliquots of standard acyclovir solution (1000 μg ml^{-1}) from 1.0 to 5.0 ml were transferred into a series of 10 ml standard flasks. To each flask 1.0 ml of F–C reagent (2 N) and 2.0 ml of Na_2CO_3 solution (20%) were added and the volume was diluted to the mark with water. The absorbance of each solution was measured at 760 nm against the reagent blank after 5 min. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

2.5.2. Tablets

Twenty tablets were weighed accurately and ground into a fine powder. An amount of the powder equivalent to 100 mg of acyclovir was weighed into a 100 ml volumetric flask, 60 ml of water added and shaken thoroughly for about 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using a quantitative filter paper. First 10 ml portion of the filtrate was rejected and 2.5 ml of the tablet extract was subjected to analysis using the procedure described above.

2.5.3. Suspension

A 5 ml aliquot of ocuvir suspension equivalent to 400 mg of acyclovir was transferred into a 100 ml volumetric flask by means of a pipette and diluted to mark with water and mixed well. A 25 ml of this solution was further diluted to 100 ml with water to obtain a sample preparation containing 1000 μg ml^{-1} of acyclovir. A 2.5 ml of this diluted solution was subjected to analysis as described under general procedure. The results are presented in Table 4.

2.6. Recovery studies

Different concentration of pure drug in three levels (150, 200 and 250 μg ml^{-1}) were added to a known fixed concentration of drug in the formulation solution previously analysed, and the total concentration of the drug was determined using the proposed procedure. The percent recovery of the added pure drug was calculated as follows:

$$\%\ \text{recovery} = [(C_v - C_u)/C_a] \times 100$$

where C_v is the total concentration of the analyte measured; C_u, concentration of the analyte present in the formulation; C_a, concentration of the analyte added to formulation.

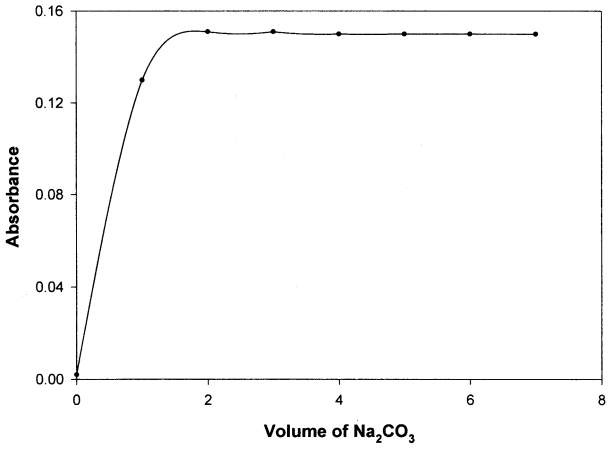

Fig. 2. Effect of sodium carbonate concentration (200 μg ml^{-1} drug + 1.0 ml of F–C reagent).

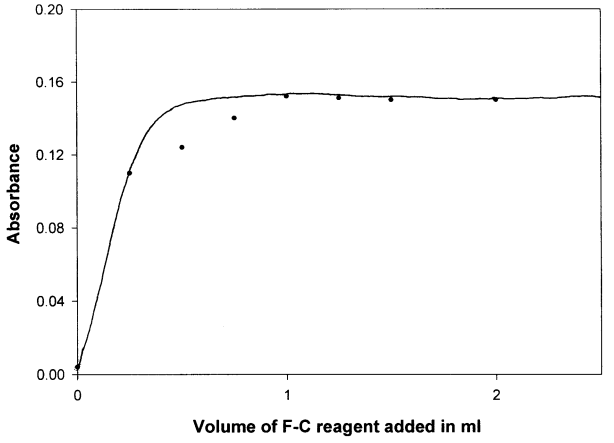

Fig. 3. Effect of F–C reagent concentration (200 μg ml^{-1} drug + 2.0 ml of 20% Na_2CO_3).

3. Results and discussion

The F–C reagent is used in the determination of many phenolic compounds [26] and a large number of substances of pharmaceutical interest [27–35]. The proposed method is based on the formation of a blue coloured chromogen, following the reduction of phospho–molybdo tungstic mixed acid of the F–C reagent [36] by acyclovir, in the presence of sodium carbonate, which could be measured at 760 nm. The colour formation by F–C reagent with acyclovir may be explained based on the analogy with reports of earlier workers [27–35]. The mixed acids in the F–C reagent are the final chromogen and involve the following chemical species:

$3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$

and

$3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$

Acyclovir probably effects reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in the F–C reagent, there by producing one more possible reduced species which have characteristic intense blue colour.

The effect of different variables such as nature and strength of alkali, optimum volumes of Na_2CO_3 and F–C reagent, reaction time and order of addition of reactants were studied and optimized for attainment of maximum colour and stability of coloured species.

3.1. Optimization of conditions, and absorption spectrum of the reaction product

Condition under which reaction of acyclovir with F–C reagent fulfils the essential requirements were investigated. All conditions studied were optimized at room temperature (32 ± 2 °C).

3.1.1. Selection of reaction medium

To find a suitable medium for the reaction, different aqueous bases were used, such as borax, sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate and sodium hydrogen phosphate. The best results were obtained when sodium carbonate was used. In order to determine the optimum concentration of sodium carbonate, different volumes of 20% sodium carbonate solution (0.5, 1.0, 2.0, 2.5, 3.0 ml) were used to a constant concentration of acyclovir (20 μg ml^{-1}) and the results of the observation are plotted in Fig. 2. From the figure, it is evident that 2 ml of 20% Na_2CO_3 solution was found optimum. Larger volumes up to 7 ml had no effect on the absorbance of the coloured species.

3.1.2. Effect of F–C reagent concentration

Several experiments were carried out to study the influence of F–C reagent concentration on the colour development and the results are shown in Fig. 3. It is apparent that 1 ml of reagent gave maximum colour and 1 ml of reagent in a total volume of 10 ml was used throughout the work.

3.1.3. Reaction time and stability of the coloured species

The colour reaction is not instantaneous. Maximum colour is developed within 5 min of mixing the reactants and is stable for at least 30 min thereafter.

3.1.4. Effect of order of addition of reactants

After fixing all other experimental variables, a few further experiments were performed to ascertain the influence of order of addition of reactants on the colour development and the results are presented in Table 1. The order of addition of serial numbers 1, 2 or 3 recommended.

Table 1
Effect of order of addition of reactants on colour development

Sl. No.	Order of addition	Absorbance [a]
1	acyclovir + F–C reagent + Na_2CO_3	0.15
2	acyclovir + Na_2CO_3 + F–C	0.15
3	F–C reagent + acyclovir + Na_2CO_3	0.15
4	F–C reagent + Na_2CO_3 + acyclovir	0.01
5	Na_2CO_3 + acyclovir + F–C reagent	0.15
6	Na_2CO_3 + F–C reagent + acyclovir	0.01

[a] For 200 μg ml^{-1} of acyclovir.

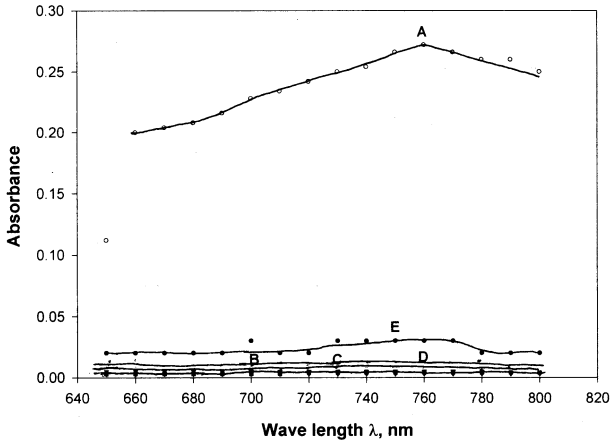

Fig. 4. Absorption spectra of: (A) reaction product (350 μg ml^{-1}); (B) pure acyclovir (350 μg ml^{-1}); (C) tablet extract (350 μg ml^{-1}); (D) suspension sent (350 μg ml^{-1}); (E) reagent blank.

3.1.5. Absorption spectra

Acyclovir reacts with F–C reagent in the presence of Na_2CO_3 to form intensely blue product with an absorption maximum at 760 nm. Fig. 4 shows the absorption spectra of the reaction product, reagent blank, acyclovir in bulk drug and acyclovir in the formulation (tablet extract and suspension). It is clear that except the reaction product no other species absorbs significantly at 760 nm. Hence, measurement at 760 nm is recommended.

3.2. Quantification

Under the described experimental conditions, a linear correlation ($r = 0.9998$) was obtained between absorbance (A) at 760 nm and concentration (C) of acyclovir over the range 50–450 μg ml^{-1}. Regression analysis of Beer's law plot gave the following linear regression equation:

$$A_{760} = 8.33 \times 10^{-3} + 6.86 \times 10^{-4} C \quad (n = 9)$$

where A_{760} is the absorbance at 760 nm and C is the concentration in μg ml^{-1}. This equation has the slope of 6.86×10^{-4} with an intercept of 8.33×10^{-3}. The standard derivation values for the slope and intercept are 5.49×10^{-5} and 1.42×10^{-5}, respectively.

The apparent molar absorptivity and Sandell sensitivity were 1.65×10^2 l mol^{-1} cm^{-1} and 1.36 μg cm^{-2}, respectively. The limit of detection was 5.68 μg ml^{-1} and limit of quantification was 18.95 μg ml^{-1}.

3.3. Accuracy and precision, and ruggedness

Precision and accuracy were found by performing seven replicate determinations containing different amounts within the Beer's law limits. The range, percent error, standard deviation and relative standard deviation (%) for seven determinations at each level are given in Table 2. The accuracy of the method is clear form the percent error values of 2.21, 1.64 and 0.10 for 100, 200 and 350 μg ml^{-1} concentration levels. The relative standard deviation values, which are less 2% for three levels studied, indicate the high reproducibility of the method. To assess the significance of the results obtained for bulk drug using the proposed procedure, a comparison of the experimental mean values ($\bar{x}$) was made with the true values (μ) using n and t values. The actual difference between the mean and the true value ($\bar{x} - \mu$) and the largest difference that could be expected as a result of indeterminate error ($\pm ts/\sqrt{n}$) are given in the last two column of Table 2. It is clear from the results that the values of ($\bar{x} - \mu$) are less than $\pm ts/\sqrt{n}$ indicating no significant difference between the mean and true values except at the concentration level of 350 μg ml^{-1}.

Table 2
Evaluation of accuracy and precision

Concentration taken (μg ml^{-1})	Concentration found [a] (μg ml^{-1})	Range (μg ml^{-1})	Error (%)	SD (μg ml^{-1})	RSD (%)	$\bar{x} - \mu$	$\pm ts/\sqrt{n}$
100	102.22	4.37	2.21	1.34	1.31	2.22	1.21
200	203.28	8.90	1.64	3.96	1.94	3.28	1.80
350	350.35	4.37	0.10	1.68	0.48	0.35	0.44

t is tabulated value; s is standard deviation and n is number of determinations.
[a] Average of seven determinations.

Table 3
Within- and between-day precision of the proposed method

Within-day			Between-day precision		
Concentration of drug taken ($\mu g\ ml^{-1}$)	Concentration found [a] ($\mu g\ ml^{-1}$)	RSD [a] (%)	Concentration of drug taken ($\mu g\ ml^{-1}$)	Concentration found [b] ($\mu g\ ml^{-1}$)	RSD [b] (%)
100.00	98.16	1.24	100.00	97.65	2.38
250.00	255.37	0.72	250.00	257.21	1.92
350.00	342.84	1.63	350.00	345.75	2.63

[a] Means and RSD values of four determinations performed on each day.
[b] Means RSD values of four determinations performed on 4 different days.

Table 4
Results of analysis of formulations containing acyclovir

Formulation	Label claim mg per tablet	Found [c] % recovery ($\pm$SD)		Student's t-value	F-value
		Proposed method	Reference method		
Acivir [a] DT tablets	200.00	98.71 ± 2.80	99.06 ± 2.22	0.27	1.49
	400.00	100.40 ± 1.50	99.28 ± 1.34	1.25	1.25
	800.00	100.33 ± 1.69	98.58 ± 0.96	2.09	3.01
Ocuvir [b] tablets	200.00	98.44 ± 1.99	99.78 ± 2.06	1.05	1.07
	400.00	101.35 ± 1.05	102.14 ± 0.86	2.96	1.49
	800.00	99.95 ± 0.66	100.38 ± 1.06	0.79	2.58
Ocuvir [b] suspension	400.00 mg per 5 ml	101.35 ± 0.99	100.10 ± 1.00	1.97	1.02

[a] Cipla India Ltd.
[b] FDC India Ltd.
[c] Average of seven determinations.

To determine the ruggedness of the method, replicate determinations at different levels of the drug were carried out. The within-day RSD values were less than 2%. The values of between-day RSD for different concentrations of the drug, obtained from determinations carried out over a period of 4 days, were within 3% and indicate that proposed method has acceptable precision. The results of the study are presented in Table 3.

3.4. Interference

Reducing ions, tryptophan, hydroxyproline, 2- and 3-hydroxypridines, ascorbic acid, uric acid also reduce F–C reagent to molybdenum blue [26]. However, these substances are seldom present in the reagents used and in the pharmaceutical formulations. Hence, the method is devoid of error due to above substances.

3.5. Application to formulations

The proposed procedure was applied to the determination of acyclovir in commercially available tablets and suspension. The same samples were analysed, simultaneously by the official method described in Ref. [3] and the proposed method. The results obtained from the two sets of analyses were compared statistically. The Student's t-values and F-values at the 95% confidence level did not exceed the tabulated values of 2.447 and 4.28, respectively (for six degrees of freedom) indicating no significant difference between the methods in so far as accuracy and precision are concerned. Table 4 summarizes these results. To evaluate the validity and reliability of the method, recovery studies were performed by standard-addition method and the results compiled in Table 5 reveal that concomitant substances such as talc, starch, sodium alginate and magnesium stearate do not interfere.

4. Conclusions

The proposed method is thus simple, rapid, precise and inexpensive, and hence can be used in routine analysis of acyclovir in bulk drug and in formulations. Though the method is not selective [26], its applicability and suitability are reflected by the recovery studies as well hundreds of substances analysed in the last few years.

Table 5
Results of recovery studies by standard-addition method

Formulations	Concentration of drug in formulations (μg ml^{-1})	Concentration of pure drug added (μg ml^{-1})	Total concentration of drug found [a] (mg ml^{-1})	% Recovery of pure drug added
Acivir DT tablets (200 mg)	98.77	150.00	245.23	97.64
	98.77	200.00	301.93	101.58
	98.77	250.00	340.52	96.71
Acivir DT tablets (400 mg)	100.40	150.00	248.56	98.78
	100.40	200.00	301.10	100.35
	100.40	250.00	354.05	100.46
Acivir DT tablets (800 mg)	100.33	150.00	253.57	102.16
	100.33	200.00	295.49	97.58
	100.33	250.00	346.13	98.32

[a] Average value of three determinations.

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