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Spectrophotometric Determination of Acyclovir Drug via Charge-Transfer Complex Formation

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ABSTRACT

An Accurate, simple, rapid, inexpensive and sensitive method has been applied for the spectrophotometric determination of acyclovir, in bulk sample and dosage form, depending on the formed charge-transfer complex between the cited drug and, 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) as a chromogenic reagent. The formed complex shows absorbance maxima at 420 nm against the reagent blank. The calibration graph is linear within (1.0 - 22.0) µg.mL-1 with a detection limit of 0.60 µg.mL-1. The results show the absence of interferences from the excipients on the determination of the drug. Therefore, the proposed method has been successfully applied for the determination of acyclovir in pharmaceutical preparations.

Keywords: Pharmaceutical, Spectrophotometric, acyclovir, Charge-transfer.

INTRODUCTION

Acyclovir is an Antiviral medication. It is primarily used for the treatment of herpes, Simplex virus infections, chickenpox, and shingles. Acyclovir, chemically known as 9-[(2- hydroxyethoxy) methyl] guanine is a purine nucleoside analogue where its chemical structure is shown in figure 1, active against herpes simplex virus type 1 and 2 and against viricella zoster virus. Acyclovir is converted by viral thymidine kinase to acyclovir monophosphate, which is then converted by host cell kinases to Acyclovir triphosphate (Acyclovir -TP). Acyclovir-TP -TP, in turn, competitively inhibits and inactivates HSV-specified DNA polymerases preventing further viral DNA synthesis without affecting the normal cellular processes [1,2].

Acyclovir has been determined in pure form, dosage forms, and biological fluids using a variety of analytical methods, including chromatography [3,4], electrochemistry [5,6], spectrofluorimetry [7,8], flow injection [9], FT-IR [10], and spectrophotometry [11-13]. The selected analytical techniques that have been reported for the Acyclovir determination depend on using some practical instrument for most of these techniques. Additionally, several spectrophotometric techniques required cooling, buffer preparation, and/or incubation reaction times to finish the reaction. Most of the reported procedures for Acyclovir determination suffer from the use of complex instruments, and the need for high expertise in their use. The unavailability of these instruments in several quality control laboratories. Accordingly, there is a need for simpler, less costly as well as easier-to-be-applied methods for the routine analysis of Acyclovir, Figure 1, as a drug of widespread use. In contrast, visible spectrophotometry is considered the most convenient analytical technique in most quality control and clinical laboratories, for the assay of different classes of drugs and metals in biological [14] and environmental samples [15] due to its simplicity, reproducibility, speed, less analysis time and reasonable sensitivity with significant economic advantages. In the present work, we developed a simple, sensitive, rapid, accurate and validated spectrophotometric method for determining Acyclovir in pure form and pharmaceutical formulations.

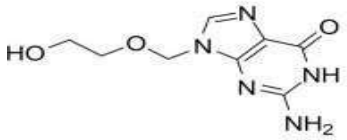


Figure 1: Structure of Acyclovir

Experimental

UV visible Spectrophotometer (Biotech UV 9200, UK) was used with 1cm quartz cuvettes to record the UV spectra of Acyclovir (ACY). A digital weighing balance (BL2105 SartoriusRomania) was used for all preparations. pH meter (AD1030 Adwa-Romania) was also used. All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Double distilled water was used throughout the investigation. The reference samples of Acyclovir were obtained from FOB, China. Sodium Hydroxide pellets extra pure AR was purchased from FOB, China. HCL was purchased from BDH company. Acivir (400mg & 800 mg) UKT was purchased from local market. A 50 mg of ACV was accurately weighed and transferred into 50 ml standard volumetric flask. To that 2 ml of 1 M NaOH was added & sonicated for 10 min. After

10 min volume was made up to the mark with the solvent to get 10^{-2} M. That solution was used as a stock solution. A 10^{2} M DDQ solution, was prepared by dissolving 0.05 g of the DDQ in 2 mL of DMF and then the solution was diluted to a final volume of 50 mL with acetonitrile. The stock solutions were freshly prepared by subsequent dilutions. This solution is prepared daily using a red-glass volumetric flask because it is a lightsensitive reagent.

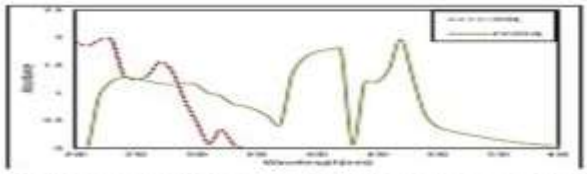
General Recommended Procedure

Measured volumes of the standard stock solution of the drug containing an appropriate amount of acyclovir were transferred into 10 mL calibrated flasks, 1 ml of 10^{-2} M DDQ solution was added to each, and then diluted to volume with acetonitrile. Absorbance measurements of resulting solutions were done at the wavelength of maximum absorption (420 nm) against a reagent blank prepared in the same manner but without the addition of Acyclovir. **Analysis of Acyclovir in Pharmaceutical Preparations**

The content of 5 tablets was mixed well and a certain amount of fine powder was accurately weighed to give an equivalent to 200 mg for tablets and dissolved in 2 ml of 1 M NaOH and 150 mL of deionized water, swirled, left to stand for 90 mints and diluted to 200mL in a volumetric flask with acetonitrile. Working solutions were freshly prepared by subsequent dilutions with acetonitrile and analyzed using the recommended procedure.

Results and Discussion

Spectrophotometric procedures are popular for their sensitivity in drug assay; hence, chargetransfer complex formation has received considerable attention for the quantitative determination of many pharmaceutical compounds [16]. Acyclovir reacts with DDQ to give red color charge-transfer complex, which exhibits absorption maxima at 420 nm against its reagent blank, Figure2. Some bands may be attributed to the formation of DDQ radical anion, which probably resulted from the dissociation of the donor-acceptor complex in relatively high polar solvents like acetonitrile [17], to avoid any interference from the reagent blank. The absorbance measurements were carried out at 420 nm in the subsequent work.



Optimization of the Experimental variables

1. Invariable Method

The experimental variables affecting the development and stabilities of charge-transfer complex formation were achieved through several preliminary experiments. Such factors include reagent volume, reaction time, pH and temperature. For this reason, a variable was modified while maintaining the other variables at their constant values, and then by maintaining this variable at its optimum value, another was modified; all variables were optimized via this method. *Effect of Reagent Volume*

The influence of the amount of the used reagent on the absorbance of acyclovir– DDQ complex is illustrated in, Figure 3. 1mL of 10^{-2} M solution of DDQ was found to be optimum to develop the maximum color intensity for formed charge-transfer complex, after which no more increase in absorbance values was obtained; therefore, the cited amount of DDQ solution was used.

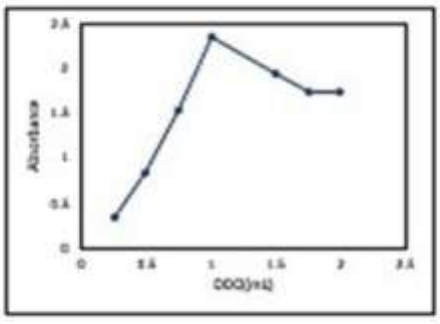


Fig. (3): Effect of reagent volume on the absorbance of 10^{-3}M Acyclovir; 10^{-2}M DDQ.

Effect of reaction time

The optimum reaction time is determined by following the colour development at ambient temperature (25 ± 2 °C). It was found that the reaction of acyclovir with DDQ, under the conditions of the study, is instantaneous, and the formed complex attained maximum absorbance immediately after mixing. The developed colour remained strictly unaltered for at least 10 min. in a dark place, Figure 4.

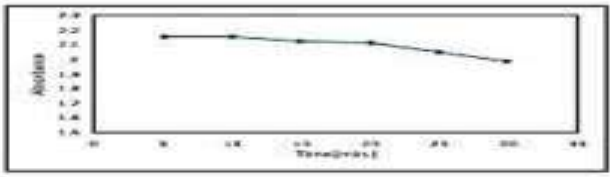


Fig. (4): Effect of reaction time on the absorbance of (10^-3 M Acyclovir; 10^-3 M DDQ).

Effect of pH

A preliminary study was conducted to demonstrate the effect of pH by monitoring the absorption of the coloured product. It was found that adding HCl or NaOH led to a decrease in absorption, so it was excluded in subsequent experiments, and all absorbance measurements were made at pH 7 as shown in Figure 5.

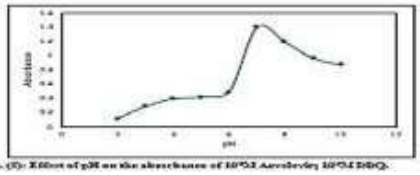


Fig. (5): Effect of pH on the absorbance of 10^-3 M Acyclovir; 10^-3 M DDQ.

Effect of Temperature

The optimum reaction temperature was determined by following the colour development at ambient temperature in the range of (20 - 60 ± 2°C). It was found that the reaction between acyclovir and DDQ is independent of the temperature of the medium up to 40°C; hence the absorbance of the complex remains, approximately, constant. The value of the absorbance starts to decrease considerably when the reaction temperature is raised above 40 °C, this may be due to the decomposition of the formed charge transfer complex. 25°C was chosen as an optimum because the product attained maximum and constant absorbance, Figure 6.

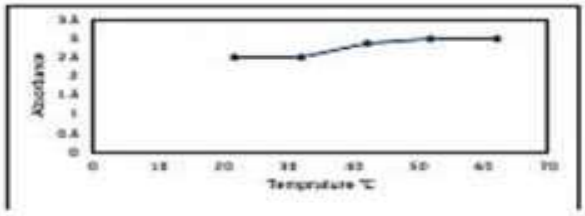


Fig. (6): Effect of temperature on the absorbance of 10^-3 M Acyclovir; 10^-3 M DDQ.

Calibration Graph

Employing the optimum experimental conditions, a linear calibration graph for the determination of acyclovir, by charge transfer complex formation with DDQ, was obtained, Figure 7, which shows that Beer's law was obeyed in the concentration range of (1.0-22.0) µg.mL^{-1}, with a correlation coefficient (R= 0.9992) and detection limit of 0.60 µg.mL^{-1}.

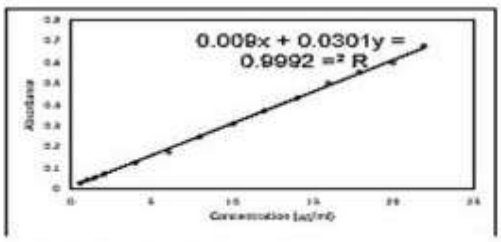


Fig. (7): Calibration graph of Acyclovir under optimum experimental conditions.

Spectral Characteristics of the Proposed Metod

Under optimum experimental conditions of the proposed method, the regression plot showed linear dependence of absorbance signals on the concentrations of the studied drug in the range given. The regression equations, correlation coefficients, molar absorptivities, detection limits and Sandell sensitivity in addition to other parameters are given in Table 1.

Table 1. Spectral characteristics and statistical data of the regression equation for determination of Acyclovir via charge transfer formation.

Parameters	Value
Color	red
Medium	pH 7
λ max, nm	420
Beers law range (µg/ml)	1-22
LOD (µg/ml)	0.60
LOQ (µg/ml)	2.009
ε (l.mole^{-1}. cm^{-1})	6778.821
Sandells sensitivity (µg/cm^2)	0.033
Regression equation: Y=bX+a	y=0.0301x+0.009
Intercept (a)	0.009
Slope (b)	0.0301
Determination coefficient (R^2)	0.9992
RSD%	0.461

Stoichiometry of the Complex

To propose a structure for the formed charge, and transfer complex between acyclovir and DDQ, two analytical procedures (Mole ratio and Job's of continuous variation method) were followed, Figures 8 and 9 respectively. The results, in both studies, showed that the complex is composed of DDQ and acyclovir with a ratio of 1:1 (DDQ: acyclovir).

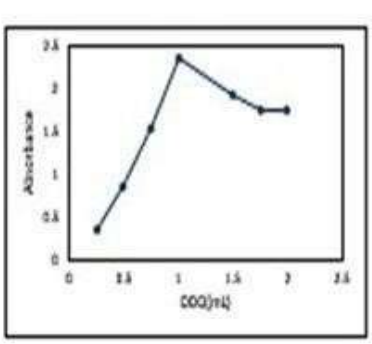


Fig. (8): Mole ratio of 10^{-4}M Acyclovir, 10^{-4}M DDQ.

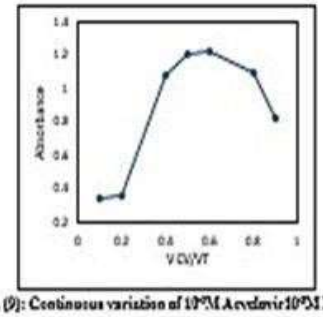
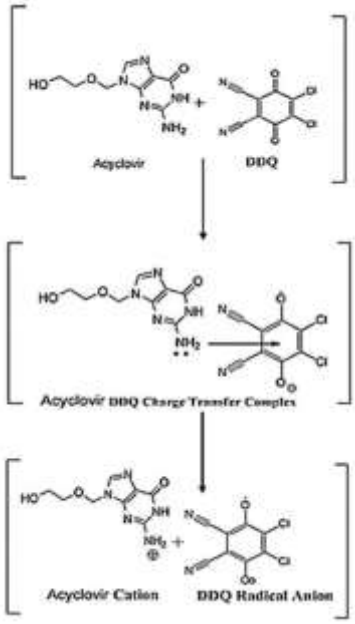


Fig. (9): Continuous variation of 10^{-4}M Acyclovir 10^{-4}M DDQ.

The structure of the formed charge transfer complex can be represented as in Scheme 1. The mechanism of the reaction depends on the formation of an original donor-acceptor (DA) complex through the interaction between one of the nitrogen atoms of amine moieties in the acyclovir (as n-electron donor) and DDQ (as πacceptor). Then, the dissociation of DA-complex may be promoted by the solvent,

especially with high ionizing power Scheme (1) such as acetonitrile, where complete electron transfer from the donor to the acceptor moiety takes place. This is followed by the formation of the DDQ radical anions as a predominant chromogen [18].



Scheme (1)

Methods	Wavelength (nm)	Beers law (µg.mL^{-1})	Molar absorptivity (L.mol^{-1}.cm^{-1})×10^4	LOD (µg.mL^{-1})
Ce(IV) in acidic medium	320	2.0-8	2.56	25.39
UV	252.8	1-20	1.5899	NA

Accuracy and Precision

The proposed method's accuracy and precision were checked by analyzing three replicates of three different concentration levels of the drug (within Beer's law range). The accuracy was determined by calculating the recovery percentage (RE%), while the precision was tested by calculating the percentage relative standard deviation (%RSD). The results indicated good accuracy with reasonable precision of the proposed method, Table 2.

Table 2. Evaluation of accuracy and precision for the determination of Acyclovir by proposed procedure.

Conc. Of ACV (taken) µg/ml	Conc. Of ACV (added) µg/ml	Conc. Of ACV (found)* µg/ml	Recovery %	Average of recovery %	RSD %
5	5	10.32	103.2	99.2	1.01
	10	14.82	98.80		0.94
	15	19.13	95.65		0.92
10	5	14.93	99.53	100.31	0.89
	10	20.0	100.0		0.95
	15	25.35	101.4		0.76
15	5	19.86	99.30	93.30	0.70
	10	24.43	97.72		0.64
	15	29.37	97.90		0.77

***** Average of three determinations

Ninhydrin-Ascorbic acid at pH 5	540	10-30	4.1071	0.3
UV	252	1.0-30	1.5899	NA
Perchloric acid-crystal violet	570	2.0-20	1.78	1.696
2,4-dinitrophenyl hydrazine (2,4 DNP)	414	20-60	NA	NA
UV	253	2-20	1.3733	NA
Cerium (IV) ammonium sulfate /3-	630	5.0-50	0.41	0.18
methylbenzothiazolin 2-one hydrazone				
Potassium sulfate /3methylbenzothiazolin 2one hydrazone	630	5.0-45	0.503	1.40
N- bromosuccinimide (NBS) / methyl orange	508	1.0-5.0	NA	0.2
Copper(II) in borax / sodium PH 9 hydroxide buffer	290	112-1620	NA	NA
Cobalt(II) in 1% pyridine in methanol	287	112-1620	NA	NA
3-methyl benzothiazoline-2one hydrazone (MBTH) /$FeCl_3$	616	20-200	0.0941	1.06
Folin-Ciocalteu (F-C) in alkaline medium	760	50-450	0.0165	5.86
Vanillin	470	2.0-10	NA	NA
pdimethylaminobenzaldeyde	404	1.81-9.06	1.10	0.024
DDQ	420	1.0-22	0 .6778	0.6

The proposed method was advantageous when compared statistically with other methods found in the literature in having good sensitivity and the results are shown in Table 3.

Table 3. Analytical parameters for the analysis of cimetidine by the proposed and others methods.

ND: Not available **Interferences Study**

The results showed that no interferences were found in the presence of up to 100, 500, 1000µg of the studied excipients (Cellulose, Microcrystalline, Sodium benzoate, Starch) in the determination of acyclovir, Table 4.

Table 4. Percentage recovery for 100 µg of Acyclovir in the presence of 100,500 and 1000 µg of excipients.

Foreign Compound	Recovery (%) of 100 µg of CV per µg of foreign compound added*		
	100	500	1000
Cellulose	95.5	102.4	100.6
Microcrystalline	97.5	95.6	98.2
Sodium benzoate	96.2	97.16	99.7
Starch	98.8	100.2	96.5

*Average of three determinations.

Analysis of Dosage Forms

The applicability of the proposed method for acyclovir determination of commercial dosage form (Tablets; CV-UKT400 mg and CVUK800 mg) was examined by analyzing the content of the active ingredient by the proposed method (charge-transfer complex formation). The results presented in Table 5, reveal that the recoveries were in the range of, reflecting the high accuracy and precision of the proposed method as indicated by low percentage relative standard deviation value. The recommended method was compared with standard addition methods.

Table 5. Spectrophotometric determination of Acyclovir in pharmaceutical preparations via chargetransfer complex formation with DDQ.

Found amount (mg)	Labeled amount (mg)	Found amount (mg)	Conc. taken (µg.mL-1)	Drug Conc. Found* (µg.mL^{-1})			
				Direct method	Recovery (%)	St. add. method	Recovery (%)
CV-	400	373.6±4	5.0	4.62	92.4	4.72	94.4
UKT400 mg							
CV-UK800 mg	800	836.8±6.4	5.0	5.27	105.4	5.19	103.8

*Average of three determinations.

The standard addition method was applied to demonstrate the efficiency and success of the proposed spectrophotometric method for the determination of Acyclovir in the pharmaceutical preparations (Tablets; CVUKT400 mg and CV-UK800 mg), and its free from interferences, A comparison and evaluation between the proposed spectrophotometric method and the standard addition method were conducted to assess the accuracy and suitability of the analytical application, as illustrated in Figure 10 and 11, it is observed that the standard addition curve for Acyclovir is parallel to the direct method curve, indicating the absence of interferences. Table 5 shows the agreement of the standard addition method with the proposed method which did not exceed the permissible values in terms of the analytical variables for both recovery %. This alignment between the standard addition method and the proposed method suggests satisfactory selectivity, indicating that the method is satisfactorily selective.

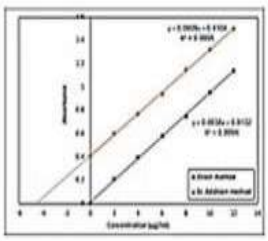


Fig. (10): Acyclovir determination by proposed method and standard addition method CV-UKT400 mg tablet solution.

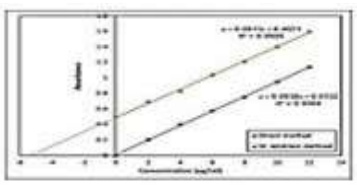


Fig. (11): Acyclovir determination by proposed method and standard addition method CV-UK800 mg tablet solution.

Conclusions

The utility of DDQ reagent for the spectrophotometric determination of acyclovir was established. The method-based charge-transfer complex formation between the cited drug and DDQ as a chromogenic reagent. The proposed method was found to be accurate, simple and sensitive. It was satisfactorily applied to the determination of acyclovir in pharmaceutical product samples.

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Conflict of interest

Authors declare no conflict of interest

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