Article

Spectrophotometric Determination of Acyclovir Drug by Azo Coupling With 4,4'-Sulfonyldianiline and Study Properties of Thermodynamic.

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Abstract.

Determination of Acyclovir in both pure and pharmaceutical preparations is carried out using a simple, accurate, and controlled spectrophotometric method. This process is done by anticreative with 4,4'-sulfonyldianiline. Optimal conditions were determined by the following factors.

In this research the detector 4,4'-sulfonyldianiline was used in addition to a suitable oxidizing agent with a basal solution of sodium hydroxide. Several factors affecting the course of the reaction have been identified and studied, including the reaction time and optimal temperature for the formation of a colored product Azo applicable to the limits of the Bear Lambert Act (2.5 to 22.5 ~ g/mL) at 620 nm wavelength. the molar absorption, Sandell's sensitivity, detection limit and quantitative limit were1048.28L.mol^{-1}.cm^{-1}, 0.3204 µg.cm^{-2}, 0.243 µg.ml^{-1} and 0.738 µg.ml^{-1} respectively.

The proposed method was successful for determining the pharmaceutical anti-drug Acyclovir in pharmaceuticals. Dynamic functions have been identified including thermal enthalpies, entropic, and caps free energy the absorption process of the drug is not automatic, the internal thermal process has made the system more regular.

Keywords: **Acyclovir, pharmaceutical formulation, spectrophotometry, 4,4'-sulfonyldianiline.**

Introduction

The drug of Acyclovir ACV and consist chemical form (9-[(2-hydroxyethoxy) methyl] guanine) Figure 1 is a synthetic purine-based nucleoside analogue that unactive herpes simplex virus in vitro and in vivo (HSV), Human herpes virus 6 (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and varicella zoster virus (VZV) (HHV-6) [1].

It is also helpful in inhibiting HSV infections in renal allograft receptors [2] and its anti-hepatitis B virus activity has been demonstrated [3].

Acyclovir's ability to act as a pseudo-substrate and bind to the viral DNA polymerase enzyme is how it carries out its antiviral effect. Acyclovir is determined

by the phosphorylation of Acyclovir to its active acyclovir monophosphate by either a viral or cellular thymidine kinase enzyme [4].

The British Pharmacopoeia (2005) provides a UV spectrophotometric method. There are a number of informal test procedures which employ a number of different methods and methods.

Several analytical methods have been proposed for the analysis of ACV in biological fluids, including spectrophotometry and fluorescence spectrometry [5], near-infrared spectroscopy [6], liquid chromatography [7], high-performance liquid chromatography (HPLC) [8], radioimmunoassay (RIA) [9], and liquid chromatography/tandem mass spectrometry (LC/MS–MS) [10]. These methods are generally time-consuming and require expensive equipment and laborious processes, such as optimization of chromatographic conditions, pretreatment of samples for HPLC analysis, and handling of radioactive waste from RIA. While electrochemical methods including LSV, cyclic voltammetry, differential pulse voltammetry (DPV), square wave voltammetry (SWV), electro photochemistry, polarization and amperometry [11-15] offer advantages such as high sensitivity, fast response, simplicity, low cost, and more environmentally friendly measurements [16].

Drug interaction pathways have also been successfully developed; studies indicate. Due to their simplicity, adequate sensitivity and low cost, spectrophotometric procedures are still used most often for routine analytical work. Some of the colorimetric methods discussed above have drawbacks, such as long response times and low selectivity for analyses. Therefore, the purpose of this research is to provide a simple and accurate colorimetric method for determining acyclovir in dose form.

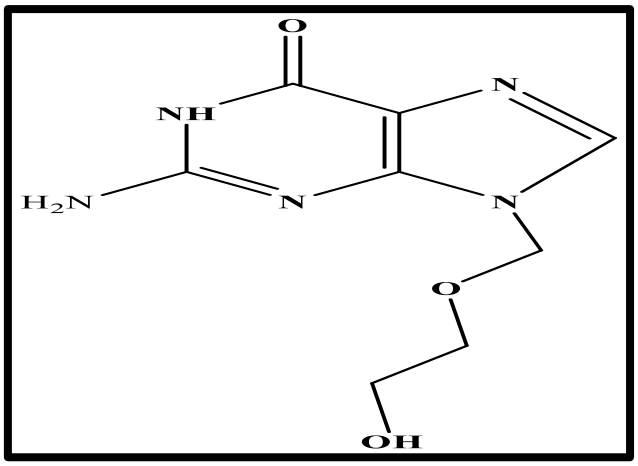

Figure (1) Chemical structure of acyclovir.

In this research, Dapsone, also known as 4,4 '-sulfonyldianiline SDA or diaminodiphenyllfone (DDS) [17], is a known antibiotic used in conjunction with rifampicin and clofazimine to treat leprosy disease. Use this drug to treat the secondary line for the treatment and prevention of pneumonia and aromatases in people with weak immune functions [18].

Also, it has been used as a treatment for acne, herpes dermatitis and various other skin diseases. [19] Dapsone is available locally and orally [20] Figure 2.

Dapsone was first discovered as an antibiotic in 1937. [19] It was initially used for leprosy in 1945. [19] It is on the World Health Organization's list of essential medicines. The model, taken orally, is available as a general and cheap medicine. [18،21]

This interaction gave a colorful response to build an easy and fast spectrometry method for determining acyclovir in the form of its dose.

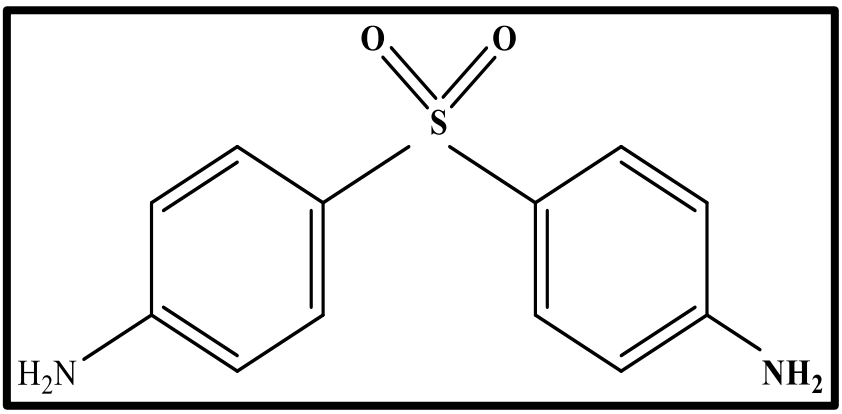

Figure (2) Chemical structure of 4,4'-sulfonyldianiline SDA.

Experimental

Apparatus

The pH measurements were made using a pH meter, an analytical balance, a water bath and a heating plate with a magnetic stirrer. Absorbance was performed using an 1800 PC UV-visible spectrophotometer Shimadzu Japan (Double Beam), withed 1 cm optical path length quartz cells.

Material and Reagent:

All chemicals were analytical in quality, and all reagent dilutions and samples were conducted with double deionized water.

Preparation of solutions

1-Standard ACV, (1000 µg. mL^{-1})

The exact weighted dose of 0.1g of the pure drug was diluted into 10 mL of concentrated sulfuric acid to produce the stock solution for ACV and the volume has been adjusted to the correct level in a volumetric vial using 100 ml deionized water.

2-ACV Working Solution (100 µg. mL^{-1})

Add deionized water to a volume bottle and dilute 10 ml of stock solution to 100 ml for preparation.

3- SDA reagent solution (5 ×10^{-4}M)

It was prepared by dissolving 0.01 gm of 4,4'-sulfonyldianiline in 2.5 mL of ethanol, Complete this volume to the mark in a 100 mL volumetric flask with deionized water.

4- Potassium periodate oxidized agent solution ($6x10^{-4}$M)

Preparation of the solution involves dissolving 0.014 gm of KIO_4 in the appropriate volume of deionized water and filling the flask to the specified volume.

5- Sodium hydroxide solution (1 M)

A 100 mL volumetric flask was used to dissolve 4 gm of sodium hydroxide in a practical amount of deionized water.

6- interference, solutions, 10000 - µg.ml^{-1}

In a volumetric flask containing deionized water, 1.0 gm of each foreign substance was dissolved before it was increased to 100 ml**.**

Initial investigations

A 10 mL graduated flask is used to create a blue product by combining 1 mL of 4,4'-sulfonyldianiline reagent (359 nm) with 1 mL of paradisiac potassium solution, Using ACV solution (332nm), add 0.5mL of sodium hydroxide to 1ml of standard ACV solution. The greatest absorption of the colored dye, compared with the white reagent, can be observed in the absorption spectrum at 620 nm**.**

Results and Discussion:

The Experimental Conditions' Optimization

For ideal conditions, the influence of various factors on the absorption intensity of 1 mL of SDA and 1 mL of KIO_4. Over 1 mL of acyclovir solution (100 g.mL-1) was examined in an alkali medium (0.5 mL) of NaOH (1 M).

Optional oxidizing agent selection

The study was performed by adding 1 mL of various oxidizing (6 x 10^{-4} M) to the SDA solution (5 x 10^{-4} M) According to the results, potassium periodate solution produces a higher intensity for colored azo dye at 620 nm than 1ml of drug solution and 0.5mLof sodium hydroxide solution. This oxidizing agent was selected in subsequent experiments due to their greater effectiveness than other oxidizing agents used, as demonstrated in table (1).

Table. 1 Effect of oxidizing factor type.

Type of oxidizing factor	absorption
4,4'-sulfonyldianiline + KIO_4 + drug + NaOH	0.422
4,4'-sulfonyldianiline + KIO_3 + drug + NaOH	0.312
4,4'-sulfonyldianiline + $NaIO_4$ + drug + NaOH	0.305

Absorption Spectra

The absorption spectrums of ACV were measured relative to ethanol. The highest peak absorption for ACV was detected at 332 nm. The absorption spectra of the product were recorded in comparison to the reagent blank after the reaction between ACV and SDA. Figure 3 shows the absorption spectra of the product compared to the reagent blank. The product was discovered to have a color and had a maximum max at 620 nm and 4,4'-sulfonyldianiline (λmax) at 359 nm, Blue shifting the (λmax) of the ACV and 4,4'-sulfonyldianiline derivatives removed any potential interference, so 620 nm was chosen for the measurements.

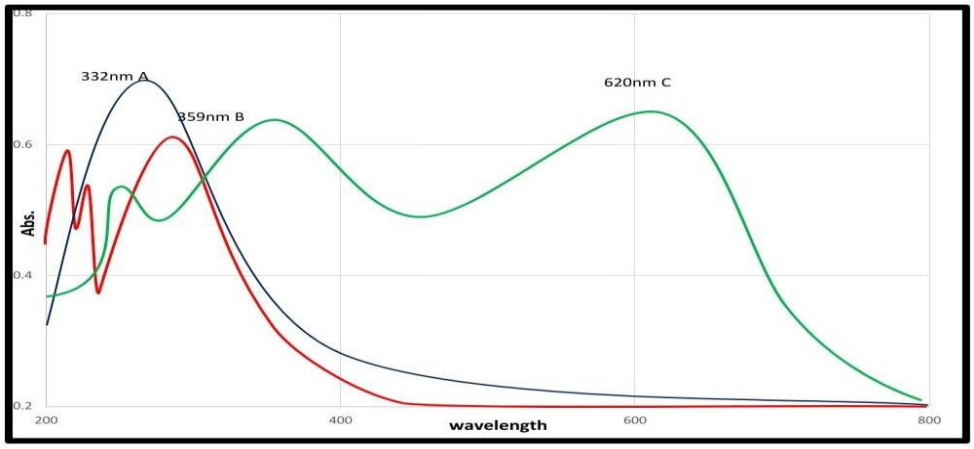

Figure (3) Absorption spectra of (A) ACV (100 µg.ml^{-1}). (B) SDA (0.005 M) (C) azo dye product of ACV with 4,4'-sulfonyldianiline.

Effect of SDA concentration

The reagent SDA was found to be inactive at a concentration of 4,4'-sulfonyldianiline ($5x10^{-5}$ to $1.5x10^{-3}$M), based on the analysis of concentrations The highest absorption obtained at the concentration ($7.5x10^{-4}$ M) of the reactant for ACV. Absorption results were unaffected by the SDA concentrations until ($1.5x10^{-3}$M) Figure 4.

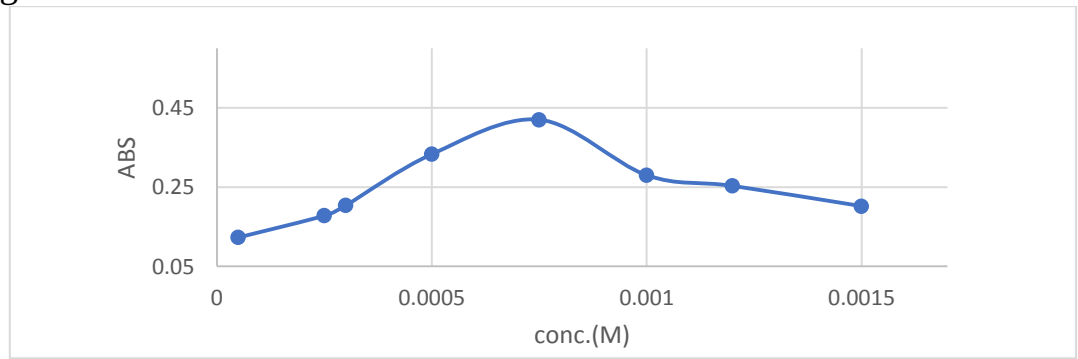

Figure (4) Effect of SDAconcentration on the azo coupling with ACV.

Effect the amount of SDA.

Using different volumes (0.25-2.5) ml of reagent at a concentration of ($7.5x10^{-4}$M) SDA for the ACV, the effect of the reagent volume on the degree of absorption of the resulting dye has been determined. At 1 mL, the highest absorption intensity was found figure 5.

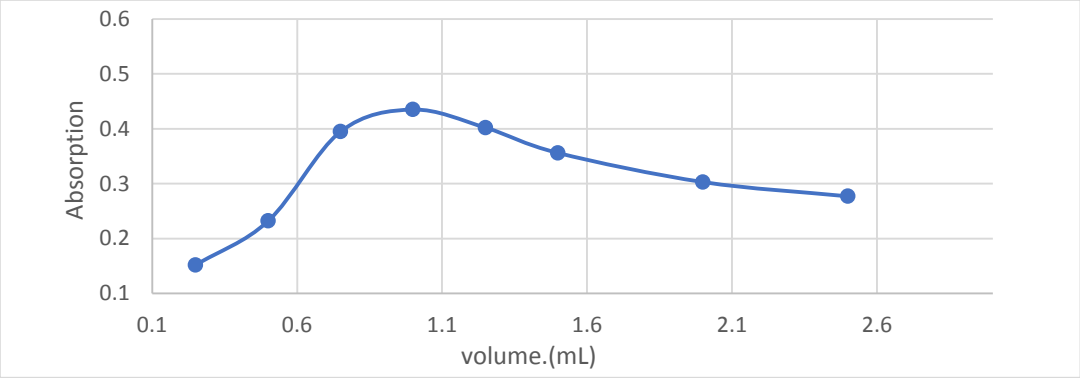

Figure (5) Effect of volume of SDA on the azo coupling with ACV.

Effect of potassium periodate concentration

By applying different KIO_4(3×10^{-4} to 2.4×10^{-3}) M concentrations and calculating the ACV wavelength-dependent uptake intensities for each solution mixture, the study investigated the impact of the oxidizing agent concentration. KIO_4 is found at ($9x10^{-4}$ M) and has the highest amount of absorption intensity. Figure 6 shows that the absorption remained unchanged despite the higher concentration of the oxidizing agent ($2.4x10^{-3}$ M) being shown.

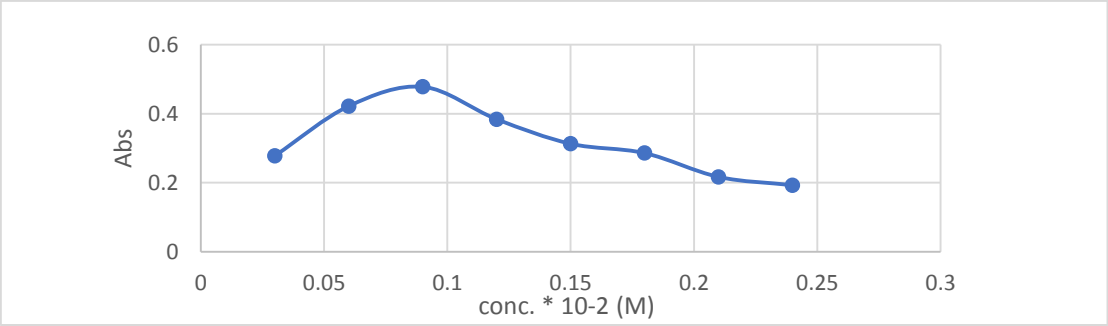

Figure (6) Effect of concentration (KIO_4) on the azo coupling with ACV.

Effect the volume of KIO_4

After determining the optimal concentration of the oxidizing agent KIO_4 ($9x10^{-4}$ M) is necessary We used volumetric flasks containing 1.0 ml of the 4,4'-sulfonyldianiline reagent ($7.5x10^{-4}$ M) and various volumes of KIO_4 (0.25 to 2.5) ml than 1.0 ml of ACV (100 g/ml) were used in the study Subsequently, 0.5 ml sodium hydroxide was added and deionized water was used to finish the volume at 10 ml. The outcomes are illustrated in Figure 7. It was determined in subsequent studies that 0.75 ml of the oxidizing agent KIO_4 was the ideal volume because it had the maximum absorbency.

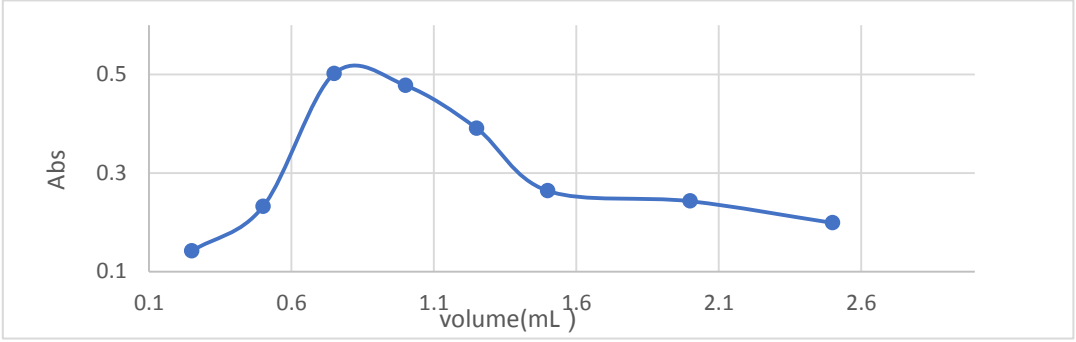

Figure (7) Effect of the Volume of (KIO_4) on the azo coupling with ACV.

Effect of volume of ACV

Using a variety of drug volumes and an ACV wavelength (100µg.ml^{-1}) to measure the intensity of absorption of the solution, the study examined the effectiveness of ACV, and found that the maximum absorption intensity was found at 1.25 mL, which is the ideal amount of ACV figure 8.

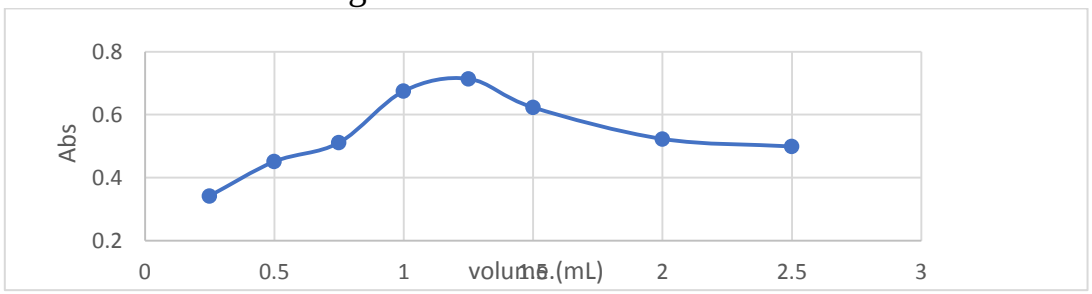

Figure (8) Effect of volume of ACV

Effect of sodium hydroxide concentration

After installing the best condition in past experiments have been the effect of studying the concentration of the basic solution on ACV. Figure 9 shows that the study was conducted using different concentrations of Sodium Hydroxide (0.25 to 1.25) M by measuring of the intensity of each solution mixture's absorption The best concentration of NaOH (0.6 M) and the highest concentration of sodium hydroxide (1.25 M) had no effect on intake values.

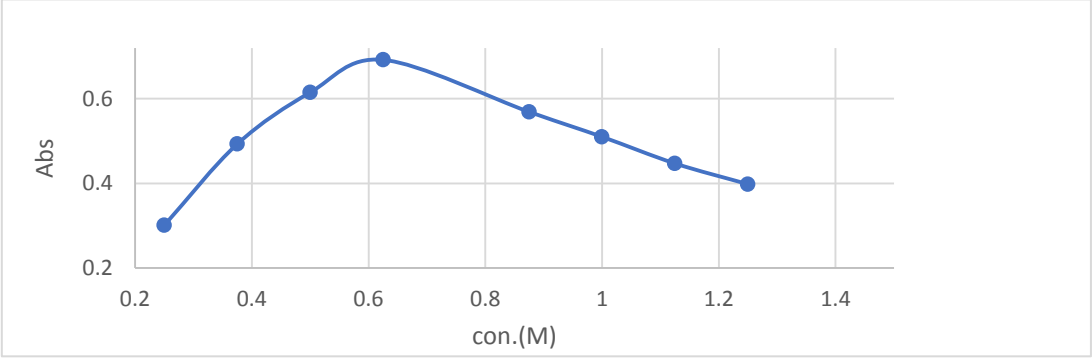

Figure (9) Effect of concentration (NaOH) on the coupling with ACV.

Effect of the volume of NaOH

In addition, the effect of the sodium hydroxide volume of ACV on the absorbency of the reaction product was also investigated. Figure 10 shows that using different volumes (0.1 to 2) ml of base solution (NaOH). The highest absorption intensity was detected the optimal NaOH volume of (0.5ml) and the higher base solution volume (2ml) did not impact the absorption values.

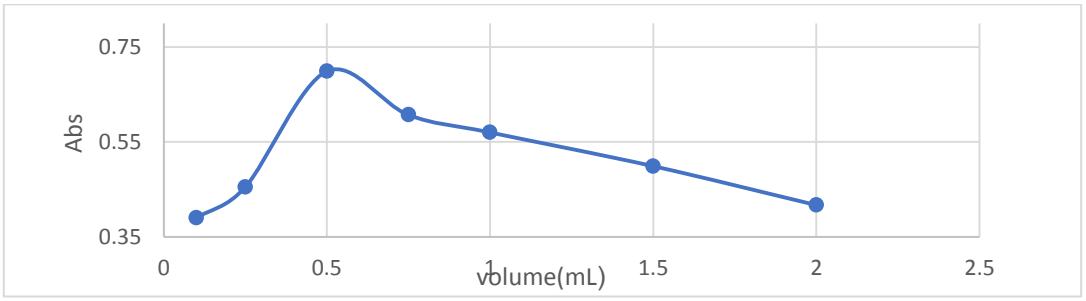

Figure (10) Effect of the volume of (NaOH) on the azo coupling with ACV.

Effect of reaction time

Adding SDA and KIO_4 to ACV for 10 minutes in basic medium resulted in a peak in color intensity after 10 minutes, which is a result of the following10 minutes is enough time for the oxidation to finish so it is used in the following studies table (2). The color was held for 65 minutes.

Table (2) Effect of reaction time Effect of temperature on reaction

Time (min)	5	10	20	30	40	50	60	70
Absorbance	0.589	0.725	0.721	0.720	0.721	0.722	0.722	0.714

Figure11 shows the results of the examination of the product's temperature (5 - 45°C) and absorbed hue show that (25°C) is the perfect temperature because it provides the best absorption. As a result, it is used in subsequent experiments.

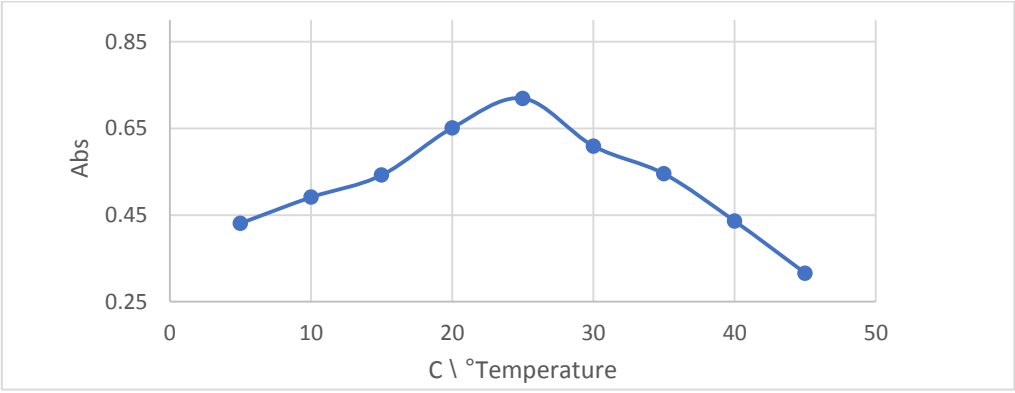

Figure (11) Effect of temperature on reaction

Sequence of additions

This investigation was conducted to determine how the product's ability to absorb color can be affected by various addition orders. Table (3) demonstrates that adding (SDA + KIO_4+ ACV+ OH^-) ensures that the colored product is fully absorbed, which is why it was used in later research.

Table (3) Addition order of ACV.

NO.	Addition	Abs
1	4,4'-sulfonyldianiline + KIO_4+Drug + NaOH	0.725
2	Drug + NaOH + 4,4'-sulfonyldianiline + KIO_4	0.287
3	4,4-'sulfonyldianiline + Drug + KIO_4 + NaOH	0.415
4	Drug+4,4'-sulfonyldianiline + KIO_4 + NaOH	0.50

Calibration curve of ACV

This figure 12 illustrates the standard calibration curve for determining ACV, which is found to follow Beer's law. The molar absorptivity was 1048.28 L/mol/cm under ideal circumstances, with a maximum wavelength of 620 nm and a correlation value of R = 0.9978. The specific absorption coefficient for the following relationship was used to calculate Sandell sensitivity at 3.204 g mL^{-2}. This analytical approach was favored to measure ACV at low concentrations due to Sandell's sensitivity and high molar absorptive value, which made it a good choice for this purpose**.**

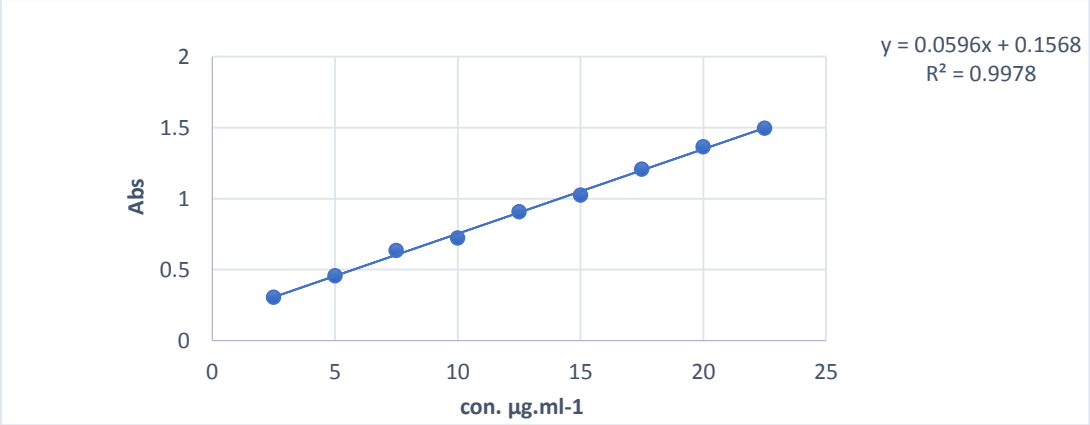

Figure (12) Calibration curve of ACV by azo coupling with 4,4'-sulfonyldianiline in presence KIO_4.

Limit of detection (LOD), linearity, and limit of quantification (LOQ)

The linearity was evaluated by creating nine ACV concentrations and evaluating it using a linear regression analysis to determine the linearity, a linear regression analysis was employed [22] Both the calibration equation and the correlation coefficient were estimated using the least square regression method within the range of (2.5-22.5) g/mL Using a linear regression analysis, the calibration curves were plotted using a comparison of concentration and absorbance. A = 0.0596 x 0.1568 acted as regression equation for results. where R is the correlation coefficient table

(4). According to the ICH guidelines, the limit of detection (LOD) ، limit of quantification (LOQ)، Relative Standard Deviation (RSD) ،Error (%) and Sandell's Sensitivity (SS) were determined to be calculated using the formulas:
LOD = 3.3 SDa / slope
LOQ = 10 SDa / slope
SDa stands for standard deviation of the blank, and b for slope [23].

$$RSD(\%) = \frac{S.D}{Mean} X\ 100$$

$$Error(\%) = \frac{Found - Taken}{Taken} X\ 100$$

$$\text{Sandell's Sensitivity(SS)} = \frac{Concentration(\mu g/mL) \times 0.001}{Absorbance}$$

Table (4) Analytic parameter for ACV determination by azo coupling with SDA in presence KIO_4.

Parameter value	Value\ACV
Beer's law, limit (µg.ml-1)	(2.5-22.5)
Molar, absorptivity (L.mol-1 .cm-1) [24]	1048.28
Sandell's, sensitivity (µg.cm-2) [25]	0.3204
Detection, limit (µg.ml-1) [26]	0.243
Quantitation, limit (µg.ml-1) [27]	0.738
Determination, coefficient(R2)	0.9978
Slope(b)	0.0596
Intercept(a)	0.1568
% RSD	%1.44
%Error	%-0.012

Accuracy and Precision for this method

Five repetitions were conducted using the proposed approach at a concentration of 5 g/mL ACV mixture with a dilution rate of 0.5 g/mL. Table (5) showed the suggested methods to calculate ACV were compared to the RSD used in the computation [28], which was found to be more reliable.

Table (5) Relative error and recovery as parameters to express the accuracy of methods determine ACV.

Values	ACV
Wavelength(nm)	620
Conc. (µg.ml^{-1})	5
X^-	0.456
%RSD	1.288%

%Error	0.334%
D.L(µg.ml^{-1})	0.326
%Recovery [29]	100.33%

The derivatization reaction's stoichiometry

To determine the ideal stoichiometric ratio of the formation reaction between reagent and ACV, Acyclovir was used at a ratio of 1:1 mole with a wavelength of 620nm. (Figure. 13 & 14)

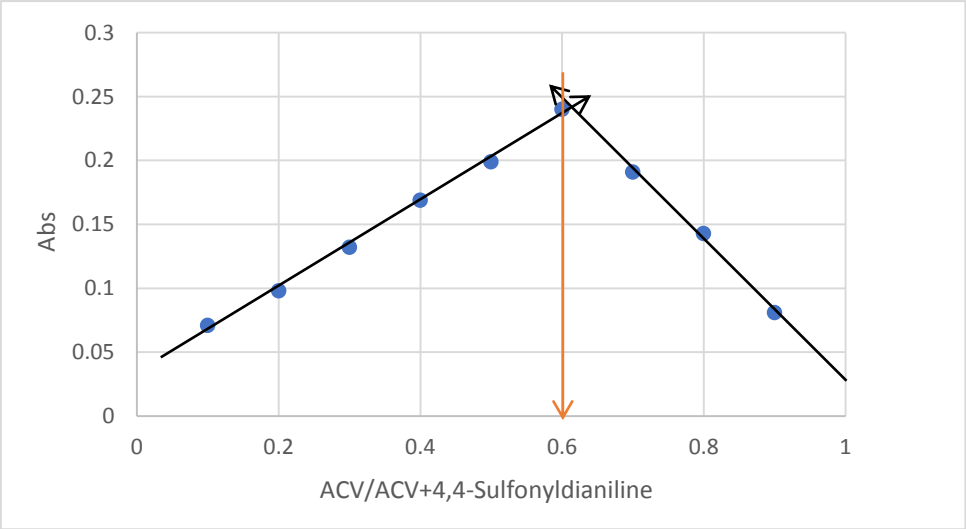

Figure (13) the continuous variation method of the reaction between SDA in presence KIO_4 with ACV.

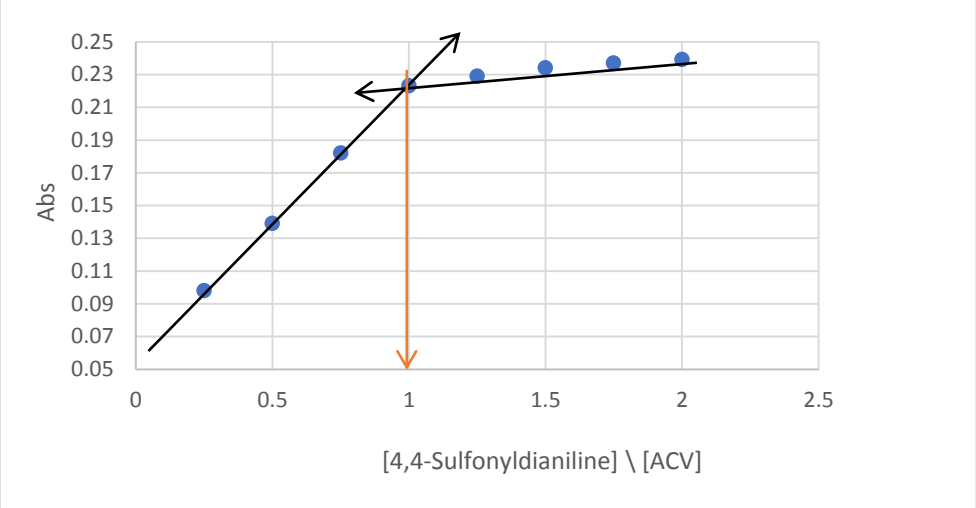

Figure (14) The mole ratio of the reaction between SDA in presence of KIO_4 with ACV.

The suggested approach to reacting SDA with KIO_4 in the presence of ACV.

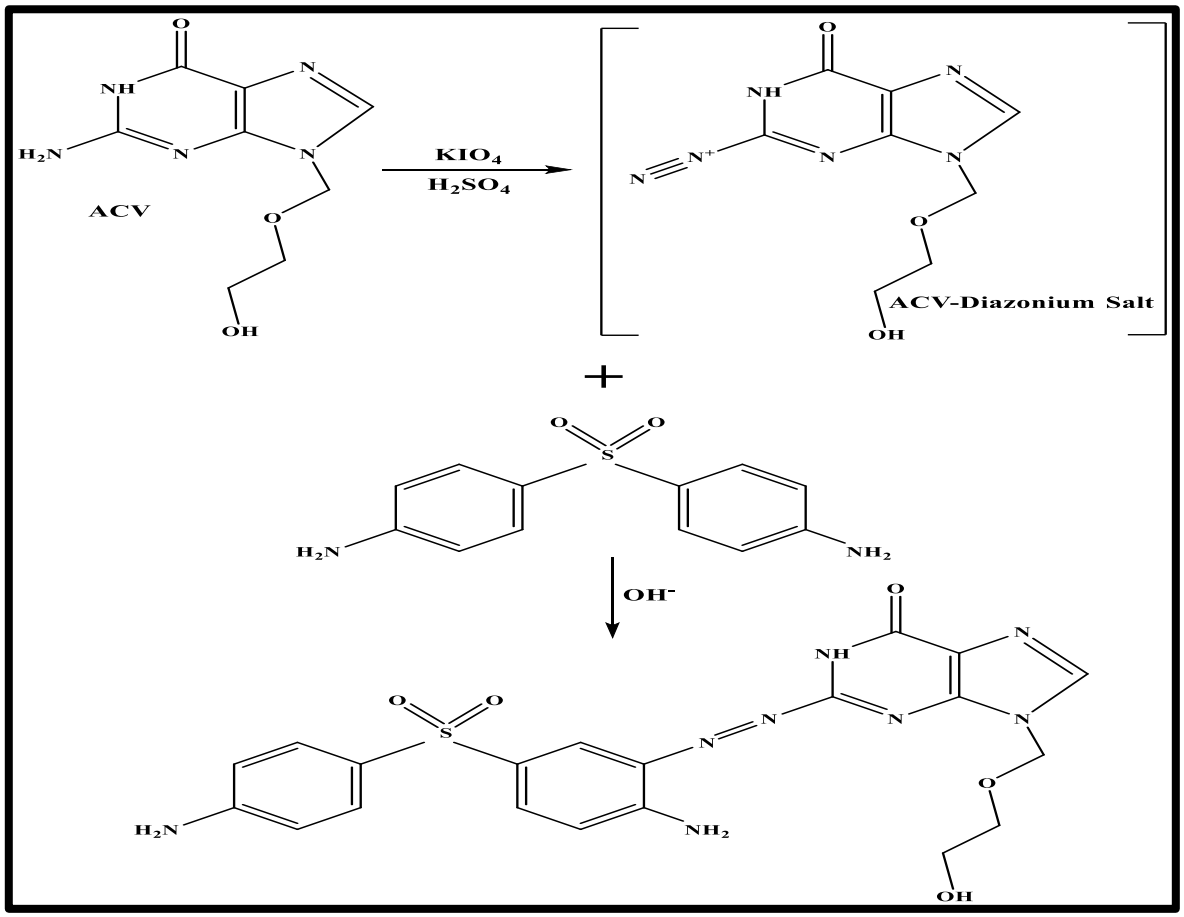

Figure (15) Scheme for the reaction pathway for determination of ACV with SDA.

Effect of interferences

The permeability method was used in pharmacological application studies to determine the effect of foreign chemical materials found in the pharmacological formula. The results are shown in table (6).

Table (6) Effect of interferences on the reaction of SDA in presence KIO_4 with ACV.

interferences	Abs
Magnesium stearate	0
Starch	0
Polyethylene glycol	0
Dibasic calcium phosphate	0
Polysorbate	0
Hydroxypropyl methylcellulose	0

Applications

Identifying the presence of ACV in the tablets used in the pharmaceutical preparation (20mg) using this method.

Into a series of 10 mL volumetric vials 1 mL of reagent 4,4'-sulfonyldianiline ($5x10^{-4}$ M) and 0.75 mL of KIO_4 ($6x10^{-4}$ M) were mixed with 1 mL of ACV solution (0.25-2.25) g.ml-1 to create the final mixture. after that is to add 0.5 ml of NaOH, Measure the absorbance after 5 minutes at a wavelength of 620nm against the blank solution after 5 minutes after diluting the contents with deionized water to the mark. The results are shown in table (7).

Table (7) analytical applications.

Sample	Conc. mg/ml		%Error	%Recovery [29]	%RSD
	Present	Found			
ACV tablets (Ireland**)**	**20**	**19.9**	**0.1**	**100.01**	**0.381%**
ACV tablets (Jordan**)**	**20**	**20.1**	**-0.9**	**99.1**	**0.455%**
ACV injection (Russian)	**20**	**20.4**	**-1.6**	**98.4**	**0.663%**

Thermodynamic study

Studying the effect of temperature allowed us to do some calculations the thermodynamic parameters (free energy change [ΔG], enthalpy [ΔH], and entropy [ΔS]. The Vant – Hoff Arrhenius equation [30]. was used to calculate (ΔH) by drawing it for the absorption of ACV. The thermodynamic parameters were calculated in this work as depicted in figure16 and table (8) with reference to the formulas.

Thermodynamic parameters: (ΔG),) and (ΔS) were found by applying these

equations:

$\Delta G = -RT \ln Keq$ ……..….(1)

$Keq = \frac{Qe\ m}{Ce\ V}$ ……..…. (2)

$\ln Keq = -\frac{\Delta H}{RT} + Constant$ ..... (3)

$\Delta G = \Delta H - T\Delta S$ ……..…..... (4)

$Slop = \Delta H$……...……...…… (5)

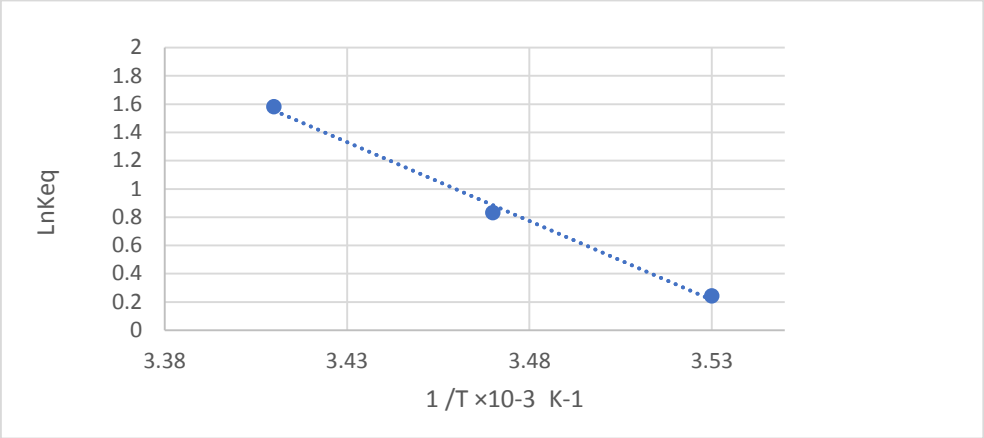

Figure (16) The relationship between ln Keq and 1/T for determination ACV by azo coupling with SDA.

Table (8) The results thermodynamic parameters of ACV.

	T (K)	ΔG (kJ/mol. K)	ΔH (kJ/mol. K)	ΔS (J/mol)
ACV	288	-0.56939	0.0927	-0.5694
	293	-1.98738		-1.9874
	298	-3.84888		-3.8489

The positive values of (ΔH) in thermodynamic parameters indicate that Acyclovir absorption is an endothermic process. [31] negative values of (ΔG) indicate, that the process is Spontaneous [32], and the negative values of (ΔS) indicate that the system is regular [33].

Conclusions

The results show that the proposed approach to the determination of ACV is simple, rapid and sensitive The process involves using the SDA reagent to oxidize with ACV in the presence of potassium periodate in a basic media to create a blue dye that is stable, water soluble, and has a maximum absorption at 620nm Identifying ACV in pharmaceuticals without temperature control or the use of organic solvents is possible through this approach, as long as the recovery is at least 100.01 per cent. The thermal dynamic properties of suggest that ACV absorption is a spontaneous, more regular and heat absorbent process, according to thermal dynamic properties.

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