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Research Article

Development of an Antiviral Drug - Acyclovir in both Pure and Pharmaceutical Forms Using a Rapid Spectrophotometric Method

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Abstract

A rapid spectrophotometric method has been described for determining acyclovir in pharmaceutical dosage forms by diazotization and coupling reaction. In this method, coupling agents such as m-dihydroxybenzene or 2-nitro-1-naphthol reacts with diazonium compound of acyclovir to produce colored azo products with maximum absorption at 462 nm and 468 nm. The calibration graph was discovered to be linear from 2.0 - 18.8 μgmL^{-1} or 3.8 – 24.2 μgmL^{-1} when diazonium compound of acyclovir coupled with m-dihydroxybenzene or 2-nitro-1-naphthol. The molar absorptivity and Sandell's sensitivity of acyclovir with m-dihydroxybenzene or acyclovir with 2-nitro-1-naphthol azo dyes were 5.399×10^4 L $mol^{-1}cm^{-1}$ or 3.600×10^4 $Lmol^{-1}cm^{-1}$ and 6.249×10^{-3} μgcm^{-2} or 9.375×10^{-3} μgcm^{-2} respectively. The results demonstrate that the reaction yielded a stable product and that the suggested approach is accurate, precise, and reasonably priced. The method works well in determining acyclovir in pharmaceutical samples.

Keywords: Spectrophotometry; Diazotization; Acyclovir; m-Dihydroxybenzene; 2-Nitro-1-naphthol

Introduction

Acyclovir is an antiviral drug [1]. It is mainly utilized for treating infections caused by the herpes simplex virus, as well as chickenpox and shingles. This medication can be administered orally, applied topically as a cream, or given via injection [2]. Additional applications include preventing cytomegalovirus infections after transplantation, and addressing severe complications associated with Epstein–Barr virus infections [2,3]. Acyclovir also known as oxopurine that is guanine substituted by a (2-hydroxyethoxy)

methyl at position 9, and a synthetic purine-based nucleoside analogue that exhibits strong inhibitory activity against varicella zoster virus (VZV), epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes virus 6 (HHV-6) and herpes simplex viruses (HSV) both *in vitro* and *in vivo* [4-7]. Acyclovir inhibits the viral DNA polymerase enzyme by functioning as a pseudo-substrate, which is how it carries out its antiviral action. The first process is the cellular or viral thymidine kinase enzyme phosphorylating acyclovir to the active acyclovir monophosphate [8-10]. Few unofficial methods such as Polarography [11], Radioimmunoassay [12,13], Near IR spectroscopy [14], Micellar electrokinetic chromatography [15], HPLC with UV detection [16-19], HPLC with MS detection [20,21], and HPLC with flourimetric detection [22-25] have been successfully developed for the drug using a variety of techniques and reaction pathways. The detection of acyclovir in pharmaceutical samples has also been carried out using techniques that rely on the drug's derivatization using chromogenic reagents [26-30], derivative [31], differential spectrophotometric [32] and spectrophotometric methods [33-37] have also been reported. For regular analytical work, spectrophotometric methods remain popular because of their affordability, ease of use, and appropriate sensitivity. Some of the aforementioned methods have drawbacks, like lengthy reaction times and poor analyte selectivity. Therefore, the goal of this study is to create a spectrophotometric method that is rapid, easy and accurate for the determination of acyclovir by diazotization and coupling reaction pharmaceutical dosage samples.

Materials and Methods

Equipment's

A JASCO V-730 spectrophotometer (Serial No. A 023561798) and pH meter (Eutech Instruments pH 510 Serial o. 1398504)

Chemicals and materials

The pure grade acyclovir (99.98%) was obtained as a gift (Cipla pharmaceutical Ltd., India) and used as a sample. A 200 μgmL^{-1} stock standard solution was prepared by dissolving the required amount of pure acyclovir in acetone. The dose samples of acyclovir were bought from a nearby pharmacy in India. Acyclovir tablets used were Acyclovir (200 mg- Welcome Healthcare), Acyclovir (400 Ranbaxy Pharmaceuticals), Acyclovir (800 mg- Paradise Pharmacy) India. Hydrochloric acid solution (0.5 M), Sodium hydroxide solution (0.5 M), Sodium nitrite solution (0.5 M), m-dihydroxybenzene or 2-nitro-1-naphthol solution (1 %).

Pure acyclovir extraction from tablet formulation (1000 μgmL^{-1})

Ten Acyclovir tablets were precisely weighed and crushed. A portion equivalent to ≈ 1g acyclovir was accurately weighed and transferred into 100 mL volumetric flasks. To extract the medication into the liquid phase, 10 mL of 0.5 M NaOH was added to the flasks, and the contents were vigorously agitated for five to ten minutes. The volume was then diluted to the mark with acetone mixed well, and filtered using a Whatman No. 42 filter paper. An aliquot of the acyclovir filtrate (100 μgmL^{-1}) was diluted to the appropriate concentrations with the solvent.

Procedure for the determination of acyclovir

A portion of the acyclovir solution (μgmL^{-1}) was placed into 10 mL calibrated flasks. After adding 1 mL of 0.5% $NaNO_2$ solution and 1 mL of 0.5 M HCl, the mixture was gently stirred and set aside to complete the diazotization reaction. After that, add 1 mL of 1% m-dihydroxybenzene or 2-nitro-1-naphthol and 1.0 mL of 1 M NaOH solutions with continuous stirring. Distilled water is then added to dilute the mixture to 10 mL. Afterwards, the absorbance at 462 or 468 nm was measured in contrast to the reagent blank.

Results and Discussion

In the presence of a base, diazonium compound of acyclovir is coupled with m-dihydroxybenzene or 2-nitro-1-naphthol to produce a coloured azo dye. The absorption spectra of the azo dye produced between acyclovir with m-dihydroxybenzene or 2-nitro-1-naphthol (Figure 1), had an absorption maximum at 462 nm or 468 nm, respectively. The plot of absorbance versus concentration of acyclovir coupled m-dihydroxybenzene or 2-nitro-1-naphthol (Figure 2) and depicts that the dyes follow Beer's law in the range of 2.0 - 18.8μg mL^{-1} of acyclovir with m-dihydroxybenzene or 3.8 – 24.2 μg mL^{-1} of acyclovir with 2-nitro-1-naphthol and Scheme 1 illustrates the reaction technique.

Effect of acid and base concentration and temperature effect

The impact of acid and base on the diazotization reaction of acyclovir (2 μgmL^{-1}) was studied by adding different acid solutions (0.5 M), such as HCl, H_2SO_4, CH_3COOH and HNO_3 and base solutions (0.5 M) such as NaOH, KOH, NH_4OH and Na_2CO_3. It

was discovered that when diazotized acyclovir was coupled with m-dihydroxybenzene or 2-nitro-1-naphthol, CH_3COOH produced low absorbance with low colour stability, HCl exhibited high absorbance with highest colour stability, and 1.0 mL of NaOH exhibited the highest absorbance. Therefore, for the acyclovir diazotization reaction, 1 mL of 0.5 M HCl and 1.0 mL of 0.5 M NaOH solutions were preferred (Table 1 and Table 2).

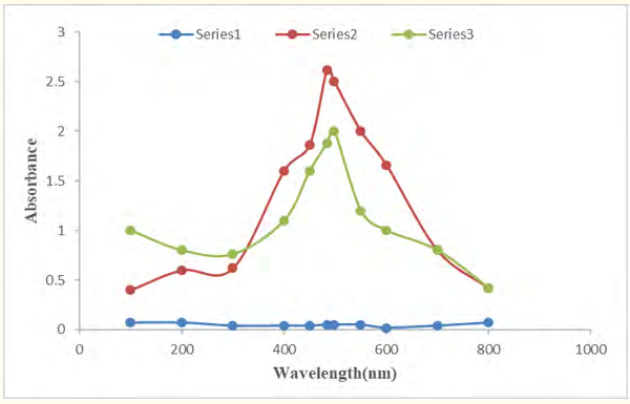


Figure 1: Absorption spectra of the azo dye produced between diazotized acyclovir with m-dihydroxybenzene against a reagent blank (Series 2) Absorption spectra of the azo dye produced between diazotized acyclovir with 2-nitro-1-naphthol against a reagent blank (Series 3) and reagent blank against distilled water (Series 1).

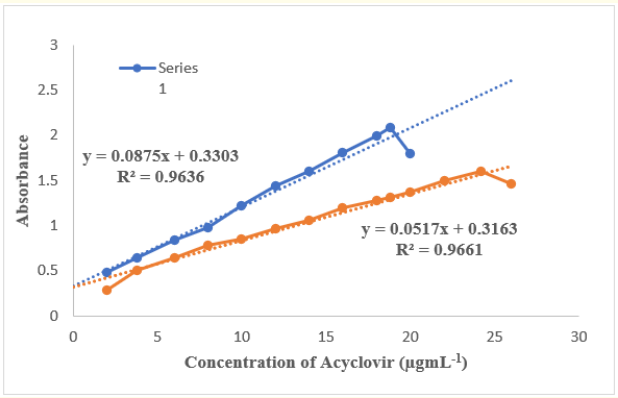
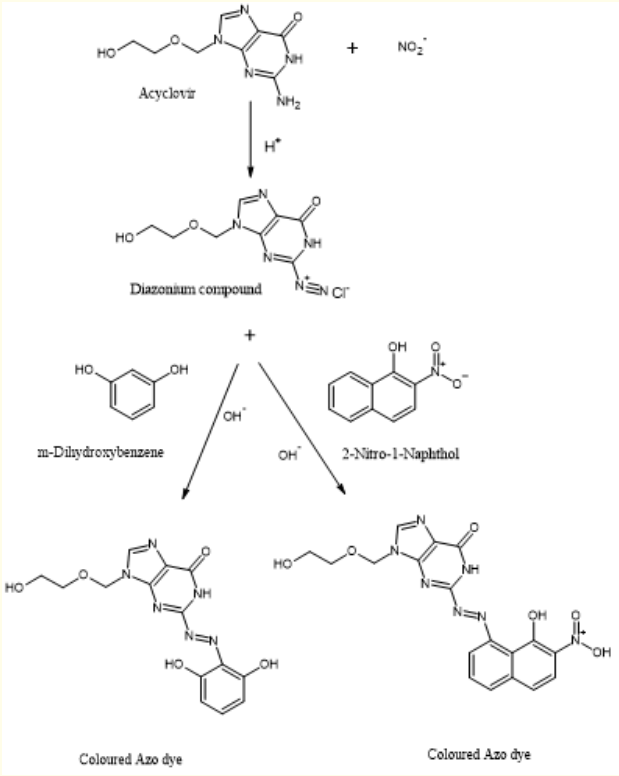


Figure 2: Adherence to Beer's law using acyclovir coupled with m-dihydroxybenzene (Series 1) or 2-nitro-1-naphthol (Series 2).



Scheme 1: Diazonium compound of acyclovir coupled with m-dihydroxybenzene or 2-nitro-1-naphthol to produce coloured azo dyes.

The influence of temperature on diazotisation processes suggests that room temperature (25 ± 5°C) is optimal, as diminished colour stability and intensity were observed at both low and high temperatures.

Effect of coupling reagents and nitrite concentration

m-dihydroxybenzene or 2-nitro-1-naphthol is used as a coupling agent by adding 0.50 to 2.0 mL of 1% m-dihydroxybenzene or 2-nitro-1-naphthol to a series of nitrite solutions. In a volume of 10 mL, it was discovered that 1 mL of m-dihydroxybenzene or 2-nitro-1-naphthol (1%) solution exhibited the brightest and firmest colour (Table 3).

Using this method with 2 μgmL^{-1} of acyclovir and adding 1 mL of 0.25-1.0 M solutions of the nitrite in hydrochloric acid (0.5 M) to a series of nitrite solutions, the colour attains its peak intensity with 1 ml of 0.5 M sodium nitrite solution. Higher concentrations did not lead in further rise in absorbance whereas lower concentrations produced unsatisfactory results (Table 4).

Effect of interference

The determination of acyclovir in the presence of various excipients such as glucose, fructose, lactose, starch and urea did not interfere with the determination of the excipients.

0.5 M acid concentration used	Absorbance (A) / mL of acid used					
	m-dihydroxybenzene			2-nitro-1-naphthol		
	0.5 mL	1.0 mL	1.5 mL	0.5 mL	1.0 mL	1.5 mL
Hydrochloric acid	0.0482	0.620	0.516	0.620	0.692	0.564
Sulfuric acid	0.424	0.468	0.404	0.600	0.642	0.582
Acetic acid	0.388	0.390	0.346	0.464	0.482	0.422
Nitric acid	0.360	0.384	0.264	0.344	0.386	0.342

Table 1: Effect of acid concentration.

0.5 M base concentration used	Absorbance (A) / mL of base used					
	m-dihydroxybenzene			2-nitro-1-naphthol		
	0.5 mL	1.0 mL	1.5 mL	0.5 mL	1.0 mL	1.5 mL
Sodium hydroxide	0.862	0.888	0.862	1.040	1.220	0.882
Potassium hydroxide	0.635	0.668	0.642	0.842	0.882	0.812
Ammonium hydroxide	0.420	0.448	0.328	0.622	0.688	0.582
Sodium Carbonate	0.248	0.288	0.204	0.461	0.520	0.442

Table 2: Effect of Base concentration.

1% m-dihydroxybenzene or 2-nitro-1-naphthol solution used (mL)	Absorbance (A) for m-dihydroxybenzene	Absorbance (A) for 2-nitro-1-naphthol
0.50	0.320	0.468
1.00	0.480	0.522
1.50	0.442	0.506
2.00	0.380	0.484

Table 3: Effect of m-dihydroxybenzene or 2-nitro-1-naphthol solution on absorbance.

1 ml of $NaNO_2$ solution used (M)	Absorbance (A)	
	m-dihydroxybenzene	2-nitro-1-naphthol
0.25	0.286	0.340
0.50	0.416	0.486
0.75	0.320	0.464
1.00	0.316	0.422

Table 4: Effect of sodium nitrite.

Analytical data

Plotting absorbance versus concentration of acyclovir, results in a straight line on the graph. Beer's law is observed between the concentrations of 2.0 - 18.8 µg mL^{-1} of acyclovir with m-dihydroxybenzene or between the concentrations of with 2-nitro-1-naphthol. The molar absorptivity of the coloured azo dye of acyclovir coupled with the diazonium salt of m-dihydroxybenzene or 2-nitro-1-naphthol was found to be 5.399×10^4 L $mol^{-1}cm^{-1}$ or 3.600×10^4 L $mol^{-1}cm^{-1}$, and the sandell's sensitivity of coloured system with nitrite-m-dihydroxybenzene or nitrite-2-nitro-1-naphthol were found to be 6.249×10^{-3} µg cm^{-2} or 9.375×10^{-3} µg cm^{-2} with maximum absorption at 462 nm and 468 nm.

The regression equation, calibration sensitivity and correlation coefficient (R^2) of acyclovir with m-dihydroxybenzene or acyclovir with 2-nitro-1-naphthol were y= 0.0875x + 0.3303 or y= 0.0517x + 03163, 0.0962 or 0.0531, 0.9636 or 0.9661 and have high dye stability ((≈ 4 h). The detection limit (D_L = 3.3/S) and quantitation limit (Q_L = 10/S) of acyclovir coupled with diazotized m-dihydroxybenzene or 2-nitro-1-naphthol were found to be 0.3431 µgmL^{-1} and 1.0395 µgmL^{-1} or 0.6215 µgmL^{-1} and 1.883 µgmL^{-1} under ideal circumstances, the better optical properties and statistical data were obtained.

Applications

This method is simple to implement, can find acyclovir in various pharmaceutical samples. The results of the suggested method align closely to the acknowledged content. For all five samples, the percentage recoveries ranged, with a 95% level of confidence, from 97.60 to 99.60 and the RSD value was 0.982 – 2.056%. The presence of pharmaceutical samples containing added ingredients had no negative effects. The results are compared with the endorsed spectrophotometric method.[33] These demonstrate that the proposed method and the recommended method are not dissimilar.

To assess precision and accuracy, multiple similar analyses were performed on five different samples that contained acyclovir at various concentrations (Table 5).

Pharmaceutical Samples	Sample taken (µgmL^{-1})	Using m-dihydroxybenzene		Using 2-nitro-1-naphthol	
		Sample found[a] (µgmL^{-1}) ± RSD	Rec. (%)	Sample found[a] (µgmL^{-1}) ± RSD	Rec. (%)
Acyclovir Sample-1 (200 mg/tab)	05.00	4.98 ± 1.727	99.60	4.82 ± 1.867	96.40
	10.00	9.80 ± 1.102	98.00	9.74 ± 1.232	97.40
	15.00	14.88 ± 1.290	99.20	14.86 ± 1.265	99.07
	20.00	19.78 ± 1.426	98.90	19.76 ± 1.114	98.80
Acyclovir Sample-2 (400 mg/tab)	05.00	4.90 ± 1.510	98.00	4.98 ± 1.840	99.60
	10.00	9.78 ± 0.982	97.80	9.88 ± 1.498	98.80
	15.00	14.76 ± 1.247	98.40	14.78 ± 1.367	98.53
	20.00	19.82 ± 1.231	99.10	19.88 ± 1.549	99.40
Acyclovir Sample-3 (800 mg/tab):	05.00	4.88 ± 2.050	97.60	4.96 ± 2.056	99.20
	10.00	9.78 ± 1.267	97.80	9.64 ± 1.929	96.40
	15.00	14.84 ± 1.226	98.93	14.78 ± 1.637	98.53
	a20.00	19.74 ± 1.540	98.70	19.80 ± 1.626	99.00

Table 5: Determination of acyclovir aarious pharmaceutical samples.

a. Mean (n = 5) ± RSD {relative standard deviation}.

Conclusions

New Coupling agents such as m-dihydroxybenzene or 2-nitro-1-naphthol, used for the spectrophotometric determination of acyclovir, are reasonably priced and selective. In comparison to various reported methods, the approach is notably straightforward, rapid, precise, sensitive, and exhibits significant dye stability (≈ 4 h). This method does not require lengthy separation or solvent extraction processes, and the high accuracy and precision of the

proposed methods are emphasized by their low relative standard deviation percentages and percentage recovery rates. The proposed methods exhibit precise, reproducible results that are free from excipient interference. The proposed method was applied to the analysis of acyclovir in pharmaceutical samples.

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Disclosure Statement

Conflict of Interest: The authors affirm that they have no conflicts of interest. Adherence to Ethical Standards: This article does not include any research involving human or animal subjects.

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