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Spectrophotometric Determination of Gancyclovir Drug by Combination Reaction with NQS as a Reagent

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Abstract. 1,2-naphthaquinone-4-sulfonate has been utilized as a reagent in the evolution of a quick and accurate spectrophotometric method for the detection of ganciclovir drugs. The process is established on the colored product formation in the basic medium between the (GCV) and the reagent. At 495 nm, the finished product's absorbance was measured. At 25 1 ℃, a reaction is carried out in an alkaline environment (PH = 9.2). The product, which is reddish orange, was made. UV-Visible spectrophotometer investigated further development of response conditions. Using Job's plot, the reaction's stoichiometry was taken into account. With a regression coefficient greater than 0.5, the established method followed the calibration curve in the concentration range (0.5 - 350 g.mL-1) (0.9965). The accuracy of the method was evaluated using the % recovery, which was found in the range (%99) and the % relative standard deviation of less than (0.2%) . The thresholds for quantification and detection were 0.545 and 1.818 g.mL-1, respectively. A successful investigation was established and validated to analyze the GCV in the tablet matrix using UV/Visible spectrophotometry. The developed inquiry was discovered to be simple and precise , therefore the suggested technique might be applied for quality control investigations in pharmaceutical metrics.

Keywords: Ganciclovir, Job plot, Spectrophotometry, NQS reagent , and maximum absorbance.

INTRODUCTION

The antiviral drug ganciclovir also known as 1,3-dihydroxy-2-propoxymethylguanine (DHPG) , is a homologue of a cyclovir and has been shown to be effective against Epstein –Barr virus , varicella-zoster virus , herpes viruses and cytomegalovirus .GCV's conversion to the triphosphate form, which prevents viral DNA polymerases from incorporating deoxyguanosine triphosphate into elongated viral DNA, is what causes it to have an antiviral effect. GCV monophosphate is incorporated into the final link in a growing chain of viral DNA after pyrophosphate is released, slowing viral replication. GCV is frequently linked to a variety of severe hematological side effects. It is crucial to keep an eye on the drug's concentration in biological fluids while receiving GCV therapy because one of this medication's side effects is a reduction of the levels of blood cells [1]. High performance liquid chromatography [2], UV-vis spectrophotometry [3], chemiluminescence [4], capillary electrophoresis [5], and voltammetric techniques [6] are just a few of the methods that have been reported for determining GCV..

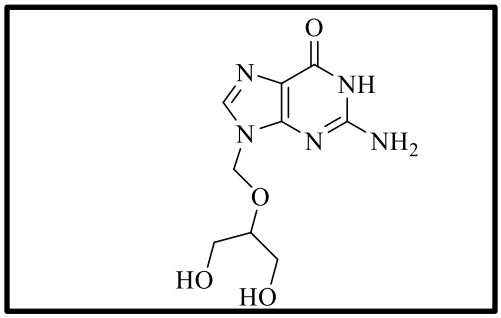

FIGURE 1. Chemical structure of Ganciclovir

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To determine drugs which are having primary and secondary amino groups by using spectrophotometric and spectrofluorometric techniques , NQS reagent is a popular reprivatizing reagent today. It is simple for these kinds of drugs to interact with NQS in an basic medium at moderate temperatures to produce a derivative product that can be effectively detected using spectrofluorometric and spectrophotometric techniques [7]. The use of the NQS for the measurement of GCV in the dosage form by using the UV/Visible spectrophotometric technique has not yet been documented in the literature. In spite of the fact that a number of UV/VIS spectrophotometric techniques have been reported for GCV estimation, to our knowledge, none of these methods used the same wavelength for GCV analysis. Therefore, the creation of a unique UV/VIS spectrophotometric technique that uses a fixed wavelength for the analysis of GCV is necessary [8]. There isn't a single report on the analysis of GCV using NQS available in the literature as of yet. thus , research in to NQS reagent was chosen in order to produce a sensitive and precise UV/Visible spectrophotometric examination for GCV analysis in the tablet matrix . Additionally, The Job's method was successful in establishing the ratio of stoichiometric of the reaction GCV and 1,2-naphthoquinone-4-sulfonate.

MATERIALS AND METHODS

MATERIALS

NQS reagent along with Working standard (GCV) were bought from Sigma-Aldrich in Mumbai.

Instrumentation

A pH meter (Model EQ 621, Equiptronics , India) , an electronic balance (Model Shimadzu AUX 120) , adouble-beam UV/Visible spectrophotometer (Shimadzu , Japan) equipped with deuterium lamp , and a thermostatically controlled water bath are included .

METHODS

Preparation of Stock Standard Solution

In order to create the stock solution , 1g of GCV was precisely dissolved in 1000ml of D.W . To get different concentrations , a suitable volume from it was further diluted with same solvent.

Preparation of Reagent

An accurate weight of (50 mg) of NQS was transferred to 10mL amber- colored volumetric flask and dissolved it in 10 mL D.W vigorous stirring 10 minutes, further, the volume was advised to be marked with the same solvent to obtained 0.5% w/v of NQS reagent solution.

Making a Buffer Solution

The buffer solution was made by dissolving 7.645g of $NaHCO_3$ and 0.945g of Na_2CO_3 in 800 mL of D.W and then increasing the volume to 1000 mL with D.W , this buffer solution is regulated at pH=9.2 [9]

The standard procedure for optimizing the ganciclovir -1, 2-naphthaquinone -4-sulfate complex

After adding (1 mL) of Na2CO3 buffer solution (pH 9.2) and (1 mL) of a 0.5% of NQS reagent (1,2-naphthoquinone-4-sulfonate) solution to the flask of calibration, a volume of (1 mL) of a solution of GCV was moved into (10 mL) of the flask. For 10 minutes, the resulting solution was maintained at 25 1 °C. The calibrated flask's volume was then brought up to the correct level using methanol. Maximum absorbance of the GCV-1, 2-naphthoquinone-4-sulfonate complex was seen in the blank solvent at a wavelength of 495 nm. Figure 2 displays the UV/Visible spectrum of the GCV-1, 2-naphthoquinone-4-sulfonate complex.

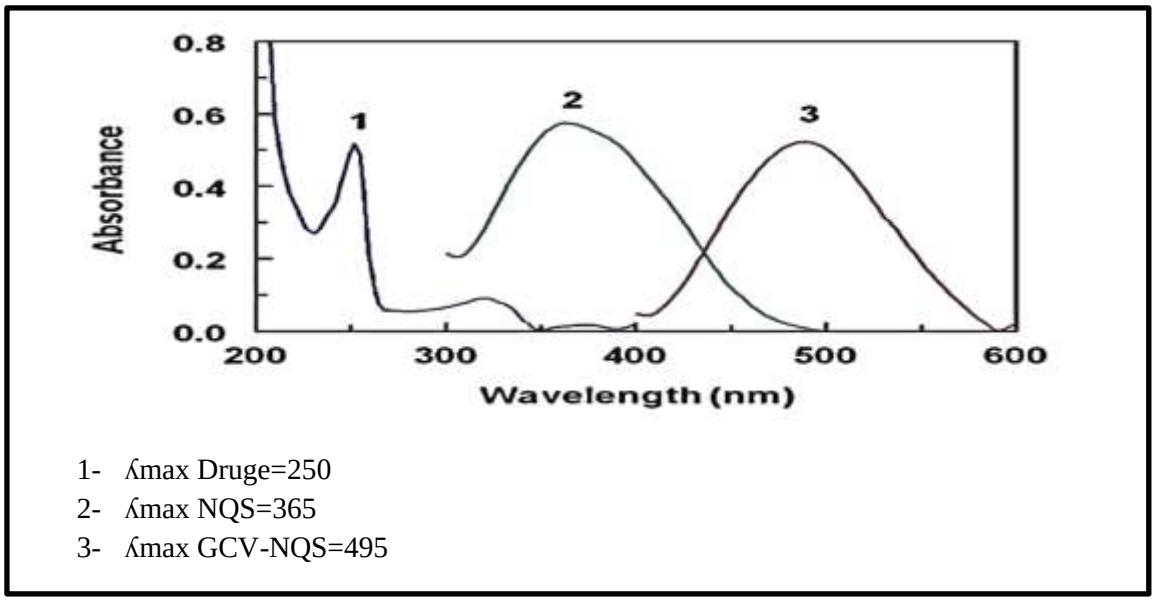

FIGURE 2. stoichiometric ratio Measurements of the of reaction between GCV and 1, 2-naphthoquinone-4-sulfonate

To estimate the stoichiometric ratio of a reaction within the GCV-NQS under operational conditions, Job's continuous variation method was successfully applied [10]. In the calibrated flask, separate aqueous solutions of GCV and NQS were made, each containing 0.002 M (2 103 M). Then, in calibrated flasks consist of (1 mL) of buffer solution, 10 mL portions of the aqueous solutions of GCV and NQS were mixed in various ratios (9:1, 8:2, 7:3,...1:9, 2:8, 3:7). (pH 9.2). Additionally, a common technique for estimating the stoichiometric proportion of response was brought closer with the subsequent arrangements.

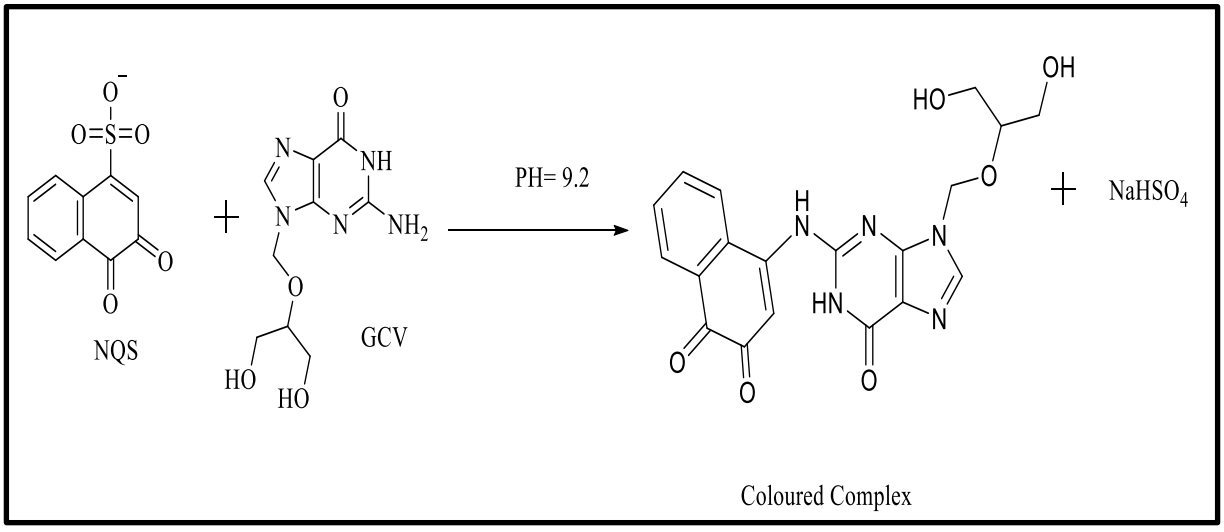

FIGURE 3. overview of reaction GCV with NQS

RESULTS AND DISCUSSIONS

Intense coloration was seen when the Ganciclovir solution was combined with NQS in an alkaline medium at room temperature, exhibiting abroad band in the region 460-545 nm . Figure 1 shows that the product is orange and has a maximum absorbance at 495nm when compared to the reagent blank , while the derivative chromogenic reagent , sodium 1,2-naphthaquinone -4- sulfonic acid , has a maximum absorbance at 365nm , indicating that formation occurs via nucleophilic displacement of the sulfonic acid group from GCV in an alkaline conditions , yielding coloured products This band became more noticeable as GCV concentrations rose.

Optimization of Reaction Condition for GCV-NQS Complex

A number of a variables , including reagent concentration , temperature , and time , must be taken into account inorder to optimize the conditions . by adjusting one variable and observing its impact on the absorbance of the colord product , the ideal conditions were found univariately.

Influence of pH of Complex Formation

With a carbonate-bicarbonate buffer, the impact of pH on the development of complex was examined over a range of pH values (9 – 10.6). Because the complex's absorbance intensity was directly correlated with the pH of the reaction medium, the factor pH of the reaction is the most requiring factor. The complex was absorbed to its maximum extent at a pH of 9.2, and its rate of absorption remained constant at a pH of 10.6. Therefore, as shown in Fig. 4, the (pH 9.2) buffer solution was utilized for the remaining analysis.

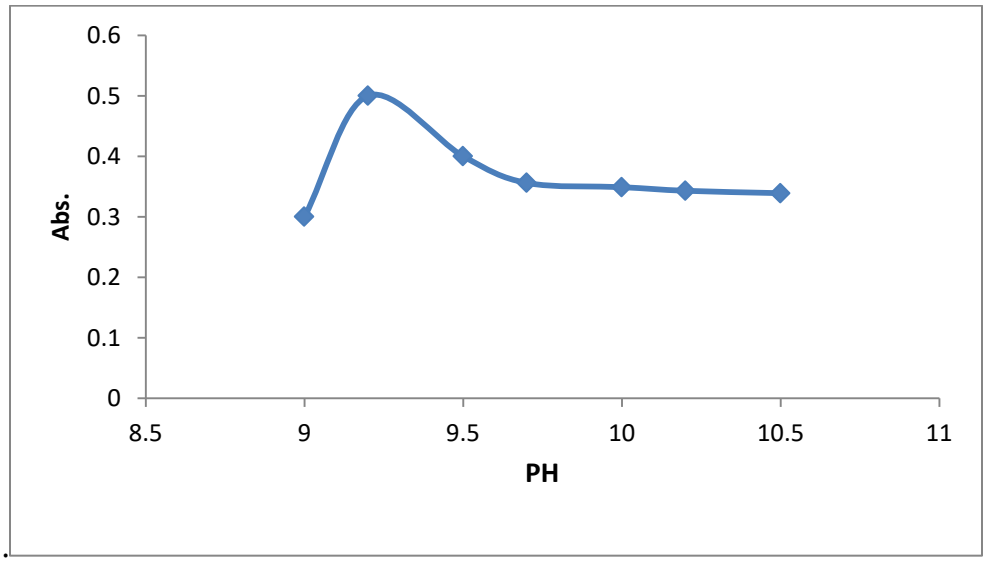

FIGURE 4. best PH

The Influence of the Concentration of NQS Reagent

When analyzing the effect of NQS concentration on the complex formation reaction , it was discovered that the complexity of the reaction was dependent on NQS concentration .when the intensity of absorbance of the complex formation reaction increased , so did the concentration of NQS .The 0.01M concentration of NQS produced excellent results . Therefore, as shown in the fig.5 , this concentration was chosen for the remainder of the analysis.

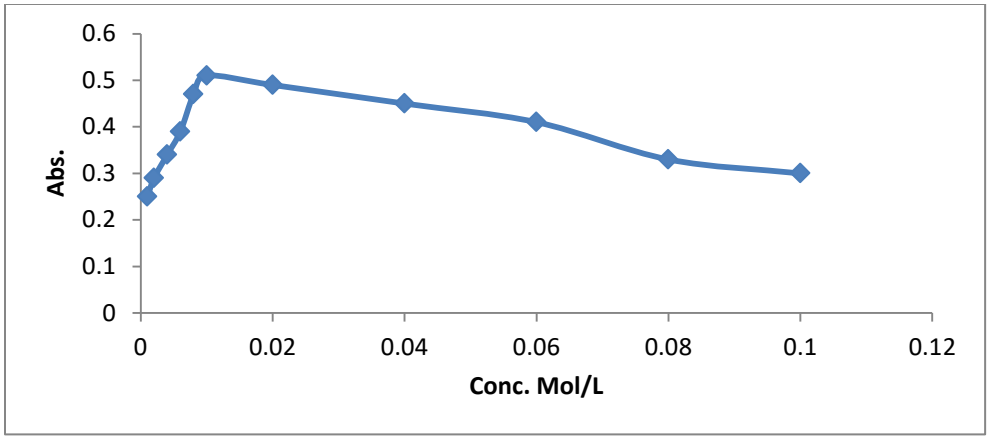

FIGURE 5. best concentration of NQS

Influence the Volume of NQS Reagent

It was discovered that the complex of reaction depend on the NQS volume while measuring the effect of NQS volume on the complex formation reaction . This results is depicted in Fig.6 . This explains why the 2mL was the ideal NQS volume.

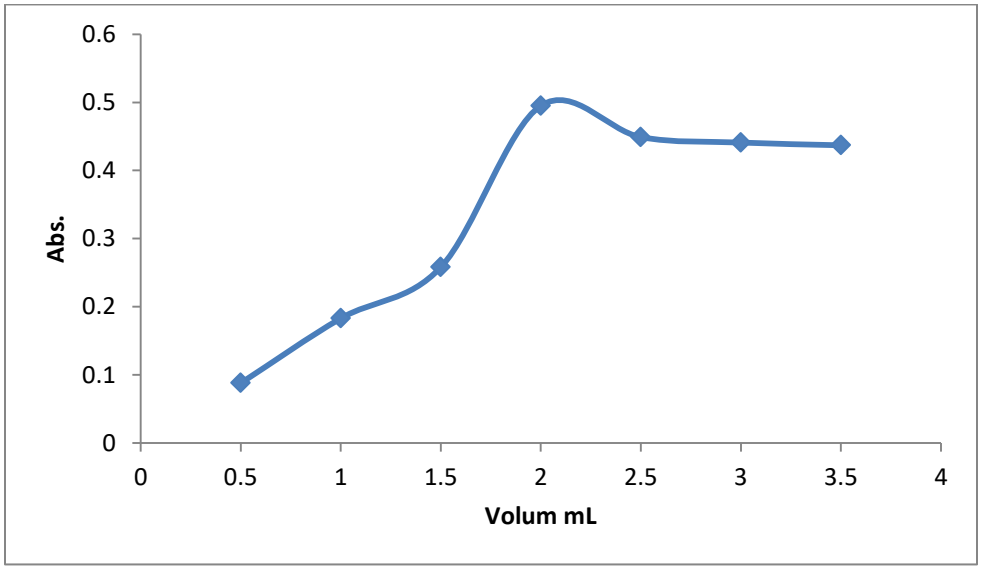

FIGURE 6. best volume of NQS

Effect of Gancyclvir Volume

The beast volume of the drugs was studied after talking different volumes from drugs GCV to measure the absorbance intensity of each solutions using the greatest wavelength. Noted that the highest optimum absorbance was at 1 mL of GCV after fixed the best concentration and volume of NQS reagent as shown in Fig. 7.

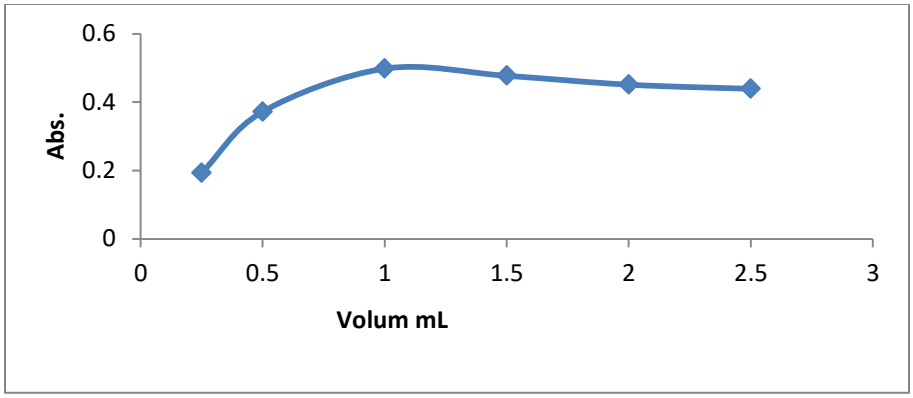

FIGURE 7. best volume of NQS

Influence of the Time

The influence of time on complex formation was examined by letting the reaction run for a range of times. The best time for the complex to form was 10 minutes as illustrated in the fig.8.

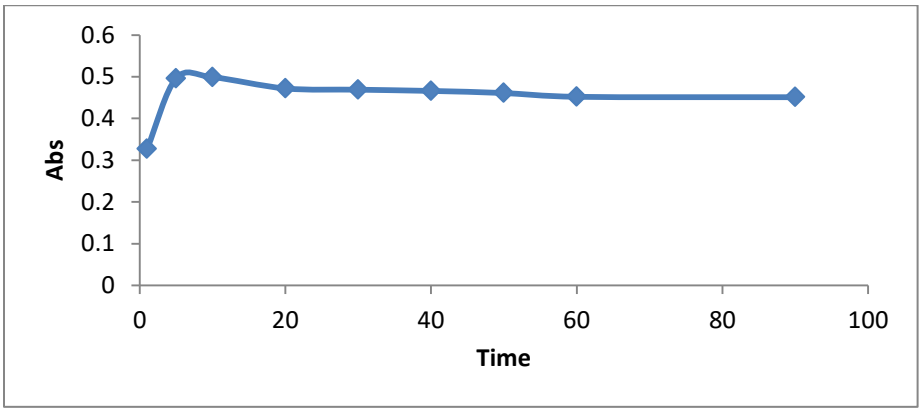

FIGURE 8. optimum time

Influence of the Temperature on the Complex Formation

The product's absorbance level was subjected to temperature testing, and the outcomes showed there was slight variation in the compound's absorbance parameters under consideration and that they were absorbent at room temperature. Therefore, as depicted in Figure 9.[11], all tests were carried out at 25 0C.

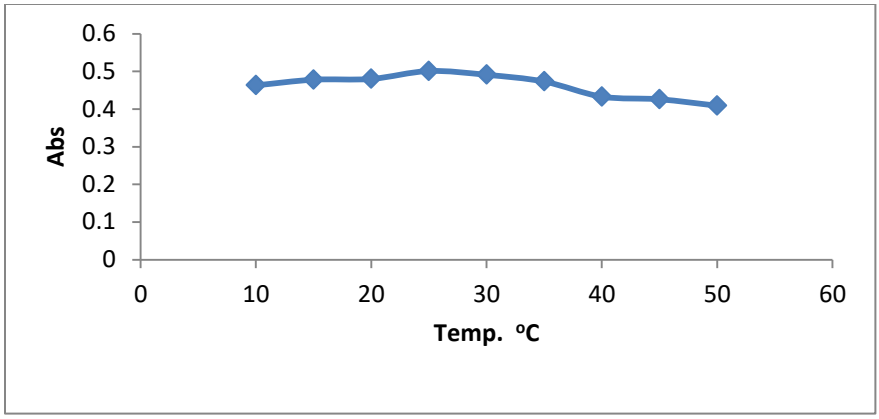

FIGURE 9. optimum time

Sequence of Addition

The sequence of introduction reaction GCV with NQS should be implemented as specified under the analytical procedure in order to achieve best outcomes, otherwise color intensity loss as well as less stability were observed (Table 1).

TABLE 1. The impact of addition order on the color absroption

No	Order of addition	Absorbance
1	Drug + Buffer +NQS	0.501
2	NQS + Drug + Buffer	0.406
3	Buffer + Drug + NQS	0.339

Statistics for the Calibration Curve

Under chosen ideal conditions, a calibration curve was created as shown in the Fig. 11. The color system in a final volume of (10 mL) (i.e., 0.5 to 350 µg/ml of GCV) is depicted on the graph, and it complied with Beer's law throughout the experiment. The proposed calibration curve's statistical data are compiled in (Table 3). 2221.39 L mol-1 cm-1 was found to be the molar absorption levels at the same time. With a sensitivity of µg cm-2, Sandell's, limits of detection and quantification were determined to be 0.185, 0.55 and 1.851 µg mL-1, respectively. Assuming ideal circumstances, various analytical specifications were obtained as shown in the (Table 4). For all three systems, the correlation coefficient demonstrated good linearity. These methods are accurate and highly sensitive, according to the values of molar absorptivity and Sandell's value [13].

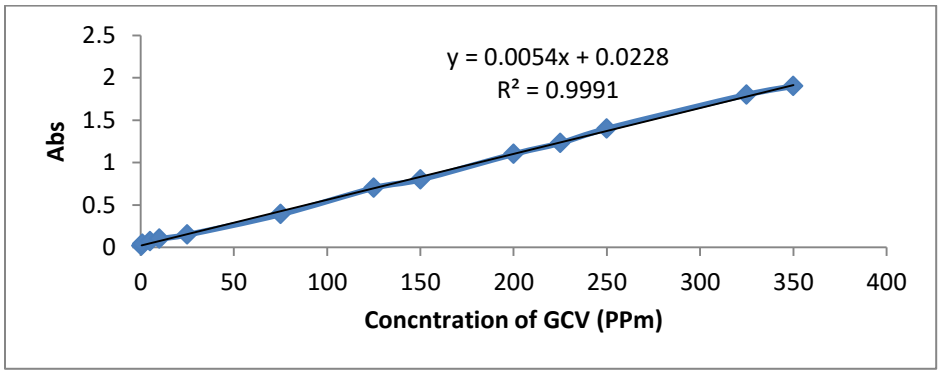

FIGURE 10. Calibration Curve

TABLE 2. Experimental abatement's analytical features

calibration curve's constant approach Parameter	Approach values
Coefficient of Correlation	0.9991
Beer's law's limitations	(0.5 – 350) (µg/ml or ppm)
Molar absorbency	0.22×10^4 (L . mol-1. cm-1)
Sandell's perceptiveness	0.185 µg . cm-2
Limits of measurement	1.851(µg/ml)
Capacity for detection	0.55 (µg/ml)
Regression formula	y = 0.0054x + 0.0228
Intercept	0.0228
Slope	0.0054
Average recovery	% 99
RSD	% 0.2

Precision And Accuracy

The relative error percentage (% Error) and relative standard deviation percentage (%RSD) were used to evaluated the precision and accuracy of the recommended GCV assessment procedure in five duplicate standard GCV solutions at three concentration levels , (Table 4) [14] contains a list of the conclusions

TABLE 3. GCV measurement in a representative sample

Samples	Claimed mg . L^{-1}	Determination of GCV by NQS system*	
1	10.0	9.85	98.5 ±0.11
2	100.0	100.11	100.13 ±0.13

* Average of five measurement

Stoichiometric Relationship and Stability Studies

This study used the stoichiometric method by continuous variation method and molar ratio (Job's method) to assess the amount of drug with NQS reagent in the base medium to formation the product in the optimum state a maximum wavelength (495 nm) where the ratio of the drug to reagent was (1:1) as shown Fig. (11) and (12).

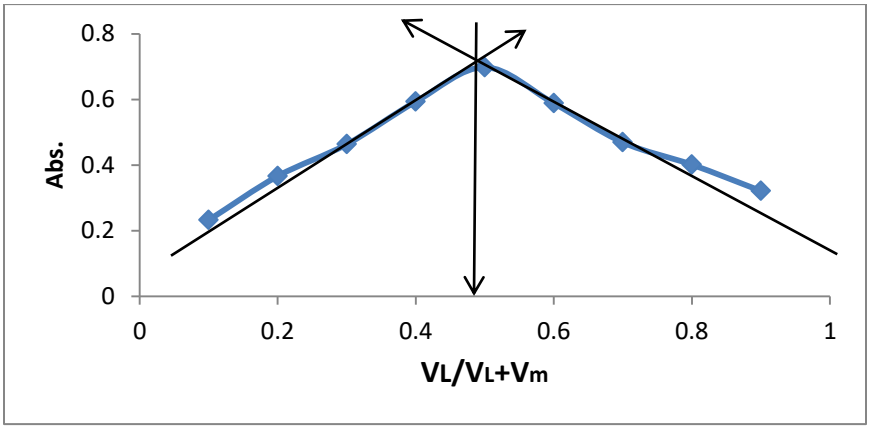

FIGURE 11. continues variation

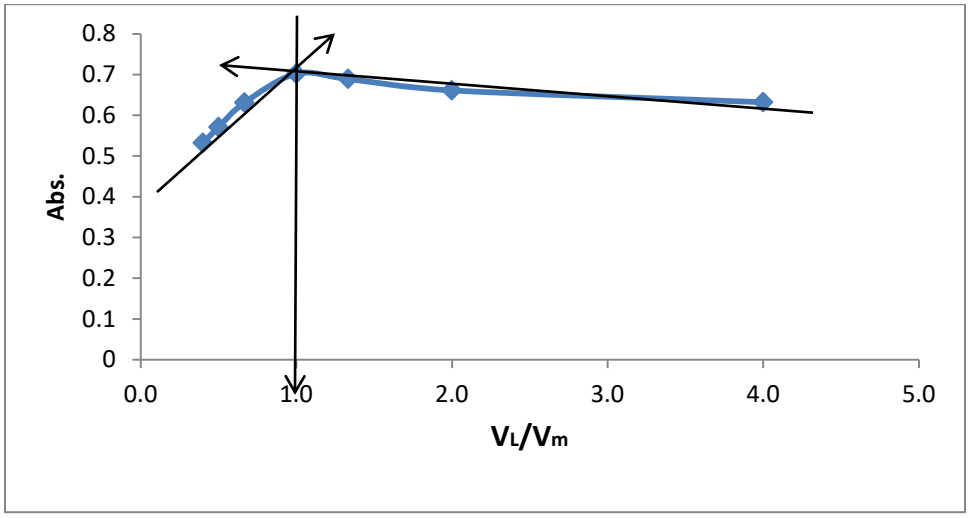

FIGURE 12. Mole ratio

Application of the Technique

The estimation of GCV in the pharmaceutical formulation was achieved with NQS by the studied analysis method. The value of %RSD, %Erel, and Recovery were calculated as show in the tables 4[15]. Also, T-test was given with no significant change, for that can consider this method is appropriate for the determination of GCV.

TABLE 4. values of %RSD, %Erel, and Recovery

sample	T- Test	Conc. Present mg. L-1 after dilution	Conc. Found. mg . L^{-1}	%RSD	%Error	%Recovery
Ganciclovir 500mg capsules/Medindia-India	0.571	100	100.3	0.2	0.3	100.3
Natclovir 250mg capsules/Wellona Pharma-India	0.190	200	198.3	0.905	-0.85	99.15

SELECTIVITY

The recovery of GCV in the presence of the excipients ranged from 96.3% to 101.9%, demonstrating the method's selectivity and demonstrating the absence of interference from excipients often employed in dosage forms.

CONCLUSION

To determine the composition of GCV in the pharmaceutical products, The current method was employed. According to the results, this approach was simple to use, quick, flexible, sensitive and simple to replicate, among other advantages. Also not needed are heating or extraction.

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