Simultaneous Estimation of Acyclovir and NicardipineHCl by UV Spectrohotometric Method with its Validation

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Abstract:

Background: Acyclovir is an antiviral drug, also known as 9-[(2-hydroxyethoxy) methyl] guanine, is a purine nucleoside analogue that prevents the replication of strains 1 and 2 of the herpes simplex virusas well as the viricella zoster virus.NicardipineHClis a dihydropyridine. It possesses a strong calcium channel blocker and a vasodilator effect.

Objectives: The current study aims to design and evaluate an easy simultaneous equation for UV spectroscopy approach for estimating both Acyclovir,NicardipineHCl in a mixture.

Methods:

Result: Result of this experiment gives value of the unknown concentration, explains linearity and range of both the drugs like for Acyclovir,y = 0.114x + 0.040,R^2 = 0.995 and for NicardipineHCl,y = 0.122x + 0.015, R^2= 0.998. The range was observed by the drug is 2 - 10µg/mL having λ_{max} at 255 nm for acyclovir and 235 nm for nicardipineHCl. The Accuracy was found to be 99-100% and precision was less than 2 giving the affirmation that method was precise and drug sample were pure. LOD and LOQ are also mention with their method of detection.

Conclusion: The purpose of this research is to develop a spectrophotometric approach for estimating acyclovir using UV spectrophotometry that was simple, quick, accurate, and specific.

Keywords: UV Spectroscopy, Acyclovir, Nicardipine, Linearity, Precision, Range, Percent Recovery, etc.

INTRODUCTION

Herpes simplex virus types 1 and 2 are prevented from replicating by the purine nucleoside analogue acyclovir, commonly known as 9-[(2-hydroxyethoxy) methyl] guanine as well as the viricella zoster virus. It prevents DNA synthesis by inhibiting the enzyme thymidine kinase. It is recognised by the USP and BP.Methods for estimating it include solid phase extraction and

HPLC2 in serum and cerebrospinal fluid, as well as electro immunoassay in serum and cerebrospinal fluid. For the measurement of medication in dosage forms, not much about spectrophotometric approach is known.[1]

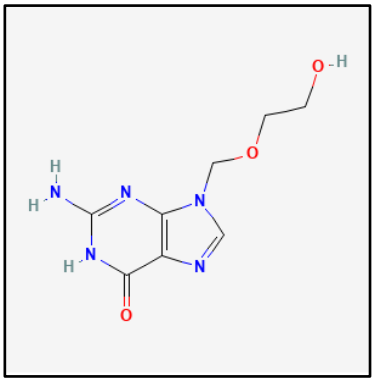


Fig 1: Acyclovir

Nicardipine Hydrochloride is a dihydropyridine. It possesses a strong calcium channel blocker and a vasodilator effect. It has antihypertensive properties and can be used to treat angina and coronary spasms while avoiding cardiotoxicity.It's also been used to treat asthma and improve the efficacy of anti-cancer medications. IUPAC of NicrdipineHCl is as follows 5-O-[2-[benzyl (methyl) amino]] 3-O-methyl two, six-dimethyl Hydrochloride of 4-(3-nitrophenyl)-1, 4-dihydropyridine-3, and 5-dicarboxylate; hydrochloride. [2]NicardipineHCl is calcium antagonist and even has potent vasodilating activity. It is quickly and fully absorbed in the digestive tract. Due to the liver's substantial first pass metabolism, plasma concentration is low.

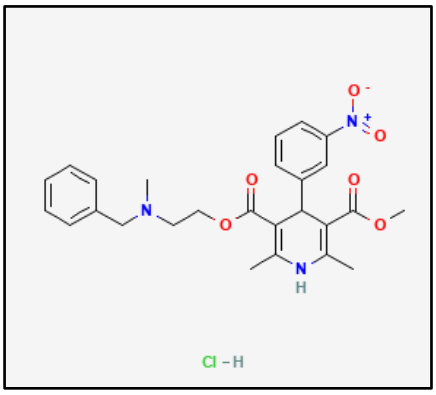


Fig 2:NicardipineHCl

As per recent article published in 2020 of the title 'Repurposing calcium channel blockers as antiviral drugs' has given an idea of enhancing the activity of antiviral drugs using calcium channel blocker. So in this article simultaneous equation of Acyclovir with NicardipineHClwas carried out. [3]

The goal of this work is to develop a straightforward, efficient, precise, and targeted UV spectrophotometric approach for quantifying acyclovir. A simultaneous UV method for measuring acyclovir and nicotinamideHCl in any formulation has not been disclosed. The goal of the current work is to develop and assess a straightforward simultaneous equation

method based on UV spectroscopy for measuring acyclovir and nicotinamideHCl in a combination.

EXPERIMENTAL WORK

Apparatus: For the development and validation of the analytical method, a double beam UV-spectrophotometer (Jasco V-630) with two 1 cm matched quartz cells, a digital balance, volumetric flasks and pipettes, and borosilicate glass beakers was employed.

Materials:
Acyclovir was purchased from Cipla Ltd. Vikhroli, Mumbai and Nicardipine was purchased from Wockhardt. Analytical grade methanol and hydrochloric acid were used for procedure.

Methods: The development and validation of the analytical method was carried out using a double beam UV-spectrophotometer (Jasco V-630), connected to computer software, and equipped with two matched quartz cells of 1 cm in length.

Melting Point Determination
Thiele's Tube was used to calculate the melting points of the two drugs.

Solubility Study (choice of solvent)
A drug's solubility was tested using Indian Pharmacopeia criteria in a number of solvents. Solubility tests were performed in both non-polar and polar liquids.[5] Methanol and 0.1 N HCL were discovered to be the most prevalent solvents for the analysis of NicardipineHCl and Acyclovir simultaneously.

UV Spectroscopy Study
Determination of Wavelength
Need actual procedure, what followed by specific concentration, volume etc.

1) **Developing a Stock Solution of Acyclovir:**

Acyclovir stock solution was created using 0.1N HCl. 100 mg of the drug were dissolved in 100 mL of 0.1 N HCl for this. Pipette 10 mL from the solution above, and then use 0.1N HCl to produce up to 100 mL. This solution yields a concentration of 100 g/mL from which a 0.1 N HCl working standard solution of acyclovir was made. From the above solution of 100µg/mL, pipette out to construct the different concentration series using Sample solutions of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1.0 mL were prepared in a 10 mL volumetric flask with 0.1 N HCl, yielding dilutions of 2 g/mL, 4 g/mL, 6 g/mL, 8 g/mL, and 10 g/mL. 0.1 N HCL was used as a solvent as a control.The same above mentioned procedure was followed for the preparation of stock solution of Nicardipine HCL in Methanol. For this, methanol was used as a blank solution.Further the series of sample of individual of Acyclovir and Nicardipine HCL were scanned through UV spectroscopy using a particular wavelength in the range of 200–400 nm. A calibration curve plot between concentration and absorbance was made in order to confirm linearity and the regression equation.

2) **Developing a Stock SolutionofNicardipine HCL** in Methanol. Take 0.1g (100mg) drug in 100mL methanol. Now remove 10mL from above solution and make up the volume to 100mL methanol. This solution results into 100µg/mL from which working standard solution of NicardipineHCl is prepared in methanol. Form the above solution of 100µg/mL removes
Sample solutions of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL are prepared in a 10 mL volumetric flask with methanol, resulting in dilutions of 2 g/mL, 4 g/mL, 6 g/mL, 8 g/mL, and 10 g/mL. Methanol is used as a blank.By using blank solution take baseline. Scan the sample solution in the entire UV-range of 400-200nm for maximum absorbance. Measure the absorbance of resulting solution at λmax under fixed wavelength measurement criteria and note down the absorbance for each solution. Plot To determine the linearity and regression equation, create a calibration curve of concentration vs. absorbance.

Simultaneous equation method:

Need actual procedure, what followed by specific concentration, volume etc.

Validation Parameters:

Validation is described as (ICH) establishing written evidence that a specific action will consistently generate the anticipated result or result in a product that satisfies the specified specifications and has the appropriate quality attributes. The method's linearity, accuracy, precision, and other qualities were examined. Limit of detection (LOD) and Limit of Quantification (LOQ) are crucial, according to ICH Q2 (R1) criteria..[4]

As we all are known about concept of validation as it is a programme with documentation that offers a high level of assurance. The primary goal of validating an analytical method is to show that it is appropriate for the intended use. The analytical producer, which controls the validation features that must be assessed, should be understood clearly. Conventional validation traits were taken into consideration in this research article they are as follows:

Linearity:

The capacity of an analytical process to produce test results that are inversely proportional to the concentration of analyte in sample is known as linearity. The proper dilution of the standard stock solution of acyclovir and nicorandil was made and examined. For both medications, the Beer-Lambert concentration range was discovered to be 2–10 g/mL. The linearity of some analytical techniques, like immunoassay, is not demonstrated after any change. In this situation, the analytical reaction should be adequately characterised by a function of the analyte concentration in the sample. It is advised to use a minimum concentration of 5 to establish linearity.

Range:

The chosen range typically results from linearity tests and is based on how the process will be used. It is proven by demonstrating that, when applied to samples containing levels of analyte within or at the extremes of the analytical method's defined range, the analytical procedure offers an acceptable level of linearity, accuracy, and precision.

In order to check whether the computer generated result are upto the mark on of the following is carried out called as **Method of least squares**. In comparison to manually created graphs, statistical analysis of the calibration data made possible by microcomputers or pre-programmable calculators offers a more elegant and precise determination of the relationship between absorbance and concentration. if there is a linear relationship between the absorbance values and concentration. The least squares approach can be used to estimate the regression line y = α - βx.

Formulas used are:

$$\boldsymbol{\alpha} = \frac{(\sum \boldsymbol{y})(\sum \boldsymbol{x^2}) - (\sum \boldsymbol{x})(\sum \boldsymbol{xy})}{\boldsymbol{N}\sum \boldsymbol{x^2} - (\sum \boldsymbol{x})^2} \quad \textbf{Equation (3)}$$

$$\boldsymbol{\beta} = \frac{\boldsymbol{N}\sum \boldsymbol{xy} - (\sum \boldsymbol{x})(\sum \boldsymbol{y})}{\boldsymbol{N}\sum \boldsymbol{x^2} - (\sum \boldsymbol{x})^2} \quad \textbf{Equation (4)}$$

Where N is the number of value pairs and y is the absorbance value at concentration x. N used is 5 for determination of equation of line.

$$\boldsymbol{r^2} = \frac{\sum(\boldsymbol{x}-\bar{\boldsymbol{x}})(\boldsymbol{y}-\bar{\boldsymbol{y}})}{\sqrt{\sum(\boldsymbol{x}-\bar{\boldsymbol{x}})^2 . \sum(\boldsymbol{y}-\bar{\boldsymbol{y}})^2}} \quad \textbf{Equation (5)}$$

Results regarding Linearity and Range are given in Table 1.

Accuracy:

The degree of agreement between the value regarded as the conventional true value or a recognised reference value and the value discovered is expressed by the analytical procedure's accuracy, which is also referred to as trueness in some cases.

The percentage of recovery from the assay of known additional amounts of analyte in the sample or the difference between the mean and the recognised actual value together with certain intervals should be used to describe accuracy. Sample analyzed with minimum three readings.

As it is output agrees with the real value. Recovery studies using the usual addition method at three distinct levels (80%, 100%, and 120%) were conducted to examine the accuracy. Standard drug solution was added to a sample solution that had already undergone analysis, and the percentage of drug content was then computed. Calculated was recovery percentage.

= [(Ct-Cs)/Ca]*100

Where, where Ct is the total drug concentration.

Drug concentration in the sample is Cs.

Drug concentration Ca is included in the sample preparation..[5]

The results are included in Table 2.

Precision:

The degree of agreement between several measurements made using different samples of the same sample under the specified conditions is referred to as the precision of an analytical method. There are three types of accuracy: repeatability, intermediate precision, and reproducibility.

Changes of outcomes across days, known as Interday, and within the same day, known as Intraday, were examined.[7]Concentration of Acyclovir and NicardipineHCl (2, 6 and

10µg/mL) these different concentration were analyzed to determine Intraday and Interday precision. These solutions was obtained by dilution for three days in a row, three times every day (intraday) (Interday). Percent RSD value 2% demonstrates the accuracy of the developed procedure.
In Table 3, results are displayed.

LOD:
The detection limit of a specific analytical method is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact quantity. Depending on whether a process is instrumental or not, there are various methods for detecting the limit of detection (LOD).

1) Visual evaluation: A non-instrumental technique for determining detection limits by analysing analysing samples with known analyte concentrations to determine the minimal detection limit.

2) Based on the signal-to-noise ratio, this method establishes the lowest concentration at which the analyte may be reliably recognised by comparing measured signals from samples with known low analyte concentrations to blank samples. In general, a signal-to-noise ratio of 3 or 2:1 is suitable for determining the detection limit.

3) Based on the slope and the response's standard deviation
The expression for the detection limit (DL) is

$$DL = \frac{3.3\sigma}{S}$$

Where,
σ the response's standard deviation is
S is the calibration curve's slope.

LOQ:
The lowest amount of analyte in a sample that can be quantitatively measured with enough precision and accuracy is the quantification limit of a specific analytical process. When determining contaminants or product degradation, LOQ (limit of quantification) is used. It can be determine by:

1) Based on visual evaluation: A non- instrumental method where quantification The minimal level at which the analyte can be quantified with respectable accuracy and precision is established after the analysis of a sample with known concentrations of the analyte.

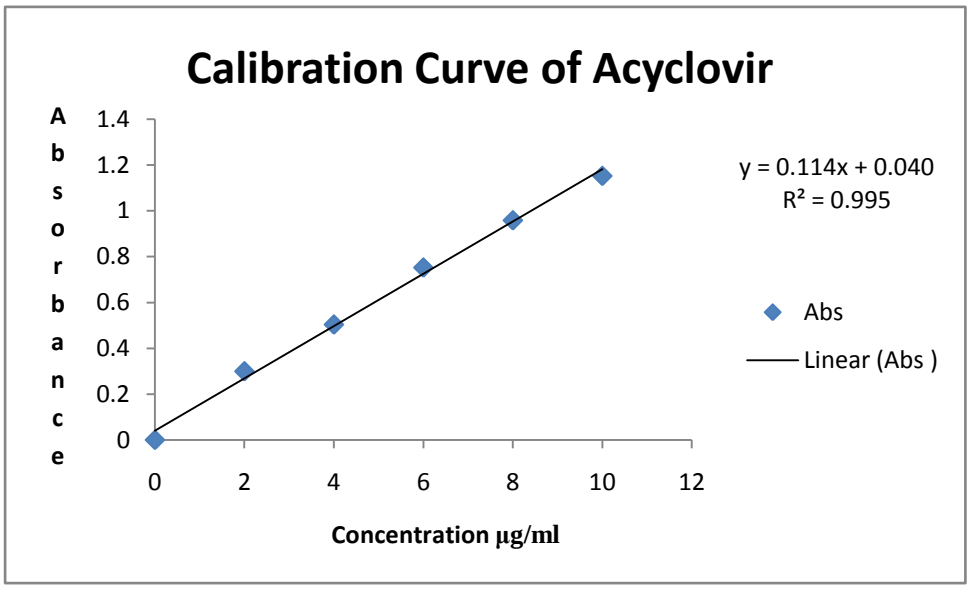
2) Based on signal- to- noise ratio: This approach can only be applied to analytical procedures that exhibit baseline noise. A typical signal- to- noise ratio is 10:1

3) Considering the slope and the response's standard deviation: You can express the quantitation limit (QL) as

$$QL = \frac{10\sigma}{S}$$

Where,
σ is departure from the response's average
S is calibration curve's slope.

Table 4 provides the LOD and LOQ results.



Conclusion and Discussion

Melting Point Determination

Melting point determination was performed by Thiele's Tube. The observation for Acyclovir was 254°C and for NicardipineHCl 182°C, respectively.

Solubility Study

UV Spectroscopy Study

For Acyclovirλmax obtained is 255nm and for NicardipineHClλmax obtained is 235nm.

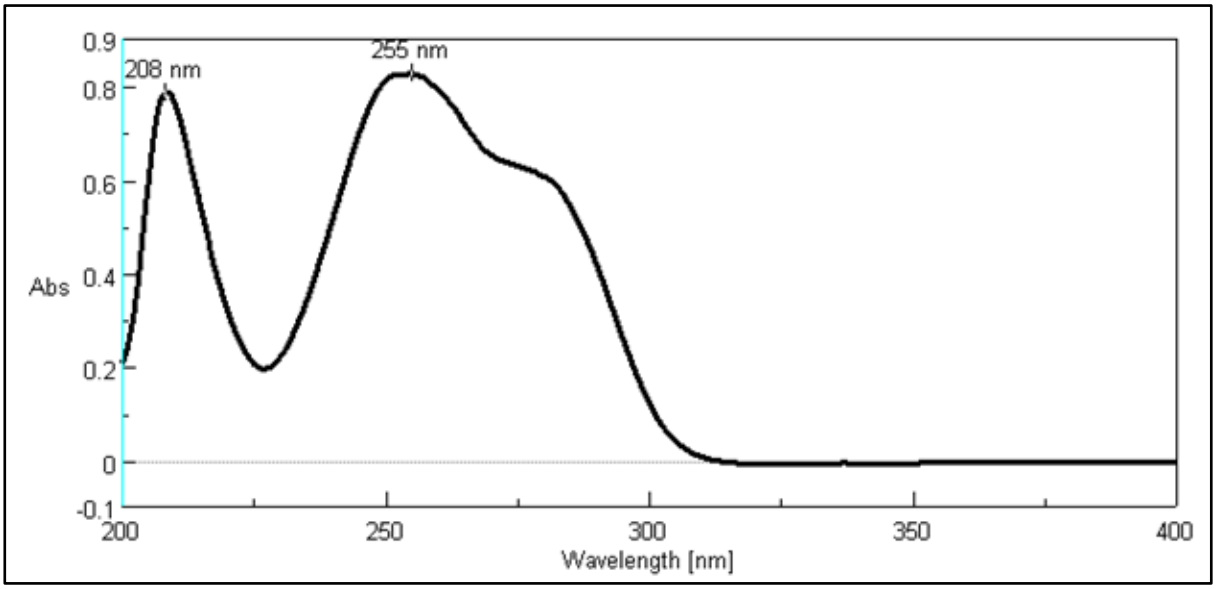


Fig 3:Acyclovirλmax Calibration curve UV

Fig 4: Calibration curve graph of Acyclovir

For NicardipineHCl

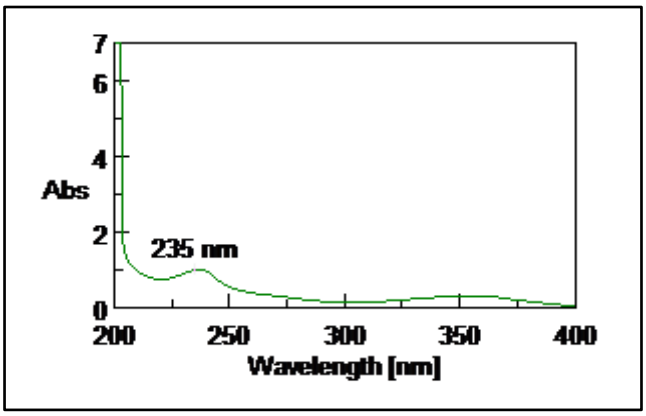


Fig 5: NicardipineHClλmax Calibration curve UV

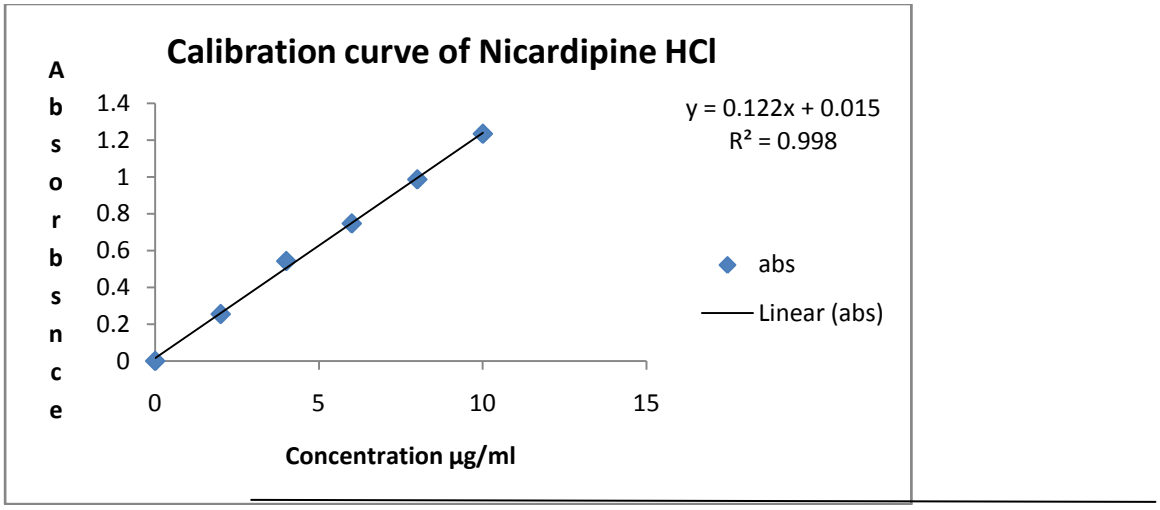


Fig 6: Calibration curve graph of NicardipineHCl

Simultaneous equation method:

To determine the maximum concentration of both medications, working standard solutions of acyclovir and nicardipineHCl were scanned in the 200–400 nm range. These wavelengths—255nm for acyclovir and 235nm for nicardipineHCl—were established. Both medications' combined spectra were captured. NicardipineHCl was used in standard solutions with concentrations of 2, 4, 6, 8, and 10 g/mL with the same dilution. By appropriately diluting their standard stock solutions into 0.1 N methanolicHCl, the concentrations of both medicines were determined at 255 and 235 nm, and Beer Lambert Law was used to calculate the absorptivity coefficients. For a certain wavelength, the graph of absorbance vs. concentration was plotted, and the regression coefficient was obtained.

The proportion of Acyclovir and NicardipineHCl were calculated by solving these simultaneous equations.

$$Cx = \frac{(A1ay2 - A2ay1)}{(ax1ay2 - ax2ay1)}$$ **.....Equation (1)**

$$Cy = \frac{(ax1A2 - ax2A1)}{(ax1ay2 - ax2ay1)}$$ **.....Equation (2)**

Where,

- ax1 = Absorptivity of Acyclovir at 255nm
- ax2 = Absorptivity of Acyclovir at 235nm
- ay1 = Absorptivity of NicardipineHCl at 255nm
- ay2 = Absorptivity of NicardipineHCl at 235nm
- Cx and Cy are concentration of Acyclovir and NicardipineHCl in the sample solution.
- A1 and A2 are the absorbance of the mixture at 255 nm and 235nm respectively.

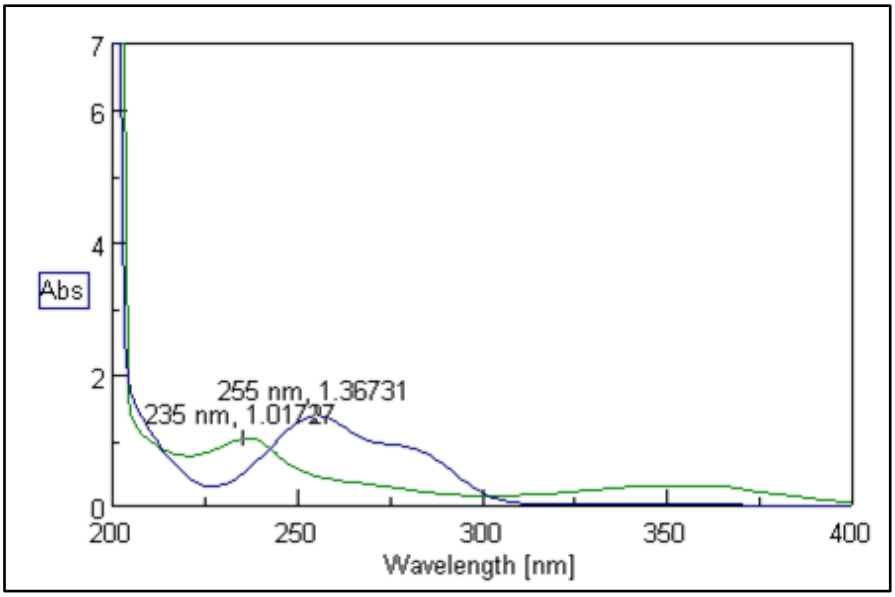


Fig 7: Overlay of Acyclovir and NicardipineHCl

Cx was found to 8 µg/mL and Cy was found to be 10µg/mL using equation (1) and (2) respectively.

Method Validation Parameters:

Discussion needed with the observed results and correlation should be mentioned between the parameters

Linearity and Range:

Table 1: Linearity and Range result.

Parameters		Acyclovir	NicardipineHCl
Linearity	**Computer generated method**	y = 0.114x + 0.040 R^2 = 0.995	y = 0.122x + 0.015 R^2 = 0.998
	Method of least square	y = 0.101x + 0.020 r^2 = 1	y = 0.110x + 0.010 r^2 = 1
Range		2 - 10 µg/mL	2 -10 µg/mL

Concentration used were 2 - 10µg/mL for both the drugs

Computer generated method gives accurate results for the linearity of drug sample of appropriate Beer Lambert range of drug.

For Accuracy:

Table 2: Accuracy data results for both the drugs.

Concentration of drug consider to be examined (in %)	**Acyclovir**	**% RSD**	**NicardipineHCl**	**%RSD**
	% Recovery ± SD		**% Recovery ± SD**	
80%	100.21 ± 0.582	0.580	99.21 ± 0.841	0.847
100%	99.78 ± 0.216	0.216	100.18 ± 0.979	0.977
120%	99.85 ± 0.124	0.124	100.32 ± 0.789	0.786

SD- Standard deviation

For Precision:

Table 3: Precision data result for both the drugs.

Drug	**Interday precision**		**Intraday precision**	
	% Amount found ± SD	**% RSD**	**% Amount found ± SD**	**% RSD**
Acyclovir	97.23 ± 0.821	0.844	99.39 ± 0.768	0.772
NicardipineHCl	98.39 ± 0.752	0.764	98.71 ± 0.981	0.993

SD- Standard Deviation

The Interday and Intraday Precision were found to be less than ± 2 % which explains this method is precise

Summary of result of Validation Parameters

Table 4: Summary of Results of Validation Parameter of Acyclovir and NicardipineHCl

Parameters	**Results**	
	Acyclovir	**NicardipineHCl**
λmax	255 nm	235 nm
Beer's law range	2 -10 µg/mL	2 -10µg/mL
Correlation coefficient (R^2)	0.995	0.998
Regression Equation	y = 0.114x + 0.040	y = 0.122x + 0.015
Slope	0.114	0.122
Intercept	0.040	0.015
Accuracy	99-100%	99-100%
Precision (% RSD)	0.772 - 0.844	0.764-0.993
LOD (µg/mL)	2.6	3.1
LOQ (µg/mL)	8.0	7.8

CONCLUSION

The created method can be summarised as simple, accurate, dependable, and cost-effective. The suggested approach was specific and free of excipients interference, so it may be used to analyse Nicardipine Hydrochloride and Acyclovir in bulk and pharmaceutical formulations on a regular basis. As combination of Acyclovir (Antiviral) and NicardipineHCl (Calcium Channel blocker) can be done under the title of repurposing of drug and using all the

characteristic of drug to prepare an effective drug product. Form the above results we can conclude that both the drugs obey Beer's range with satisfactory results for linearity and precision which was observed below 2. The validation parameters results showed that are acceptable for considering the combination of both the drug together. Formulation of any dosage form with both drugs can be prepared after studying all the compatible parameters and by determining the pharmacological result for the same.

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REFERENCES

1)Preeti Gandhi, NilofarMomin, SudhaKharade, N.P. Konapure, B.S. Kuchekar, Spectrophotometeric estimation of Acyclovir in Pharmaceutical Dosage forms, *Indian J. Pharm.Sci*., 2006;**68**(4): 516-517.doi:10.4103/0250-474X.27833

2) S.M. AL-Ghannam, A.M. AL-Olyan, Spectrophotometric determination of Nicardipine and Isradipine in Pharmaceutical formulations, *Chemical Industry and Chemical Engineering Quarterly*. 2009; **15**(2): 69-76.doi: 10.2298/CICEQ0902069A

3) VijayashreePriyadharsiniJayaseelan and ArumugamParamasivam, Repurposing calcium channel blockers as antiviral drugs, *J. Cell Commun. Signal*.2020; **14**(4):467-468.doi: 10.1007/s12079-020-00579-y

4) AmalaMateti, KiranAarelly, Manish Kumar Thimmaraju, N. Raghunandan, Method development and validation of nicardipine hydrochloride in bulk and formulation using UV spectrophotometric method, *Journal of Chemical and Pharmaceutical Research*, 2012;**4**(7):3688-3694.

5) ShilpaChaudhari, AmatulMannan and ShubhankiDaswadkar, Development and Validation of UV Spectrophotometric Method for simultaneous Estimation of Acyclovir and Silymarin in Niosome formulation, *Der. Pharmacia Lettre*, 2016; **8**(5): 128-133.

6) D. Jothieswari et al, A validated UV Spectrophotometric Method for the simultaneous estimation of AmoldipineBesylate, Valsartan and Hydrochlorothiazide in bulk and in combined tablet dosage from, *Journal of Pharmaceutical and Biomedical Sciences*,2010;**5**(5)

7) Shah U. and Gandhi A. Q- Absorption Ratio and Simultaneous equation spectrophotometric methods for Simultaneous estimation of Fenbendazole and Niclosamide in Pure Drug and Pharmaceutical formulation, *Indian Drugs* 2016;**53**(1):47-53.doi: 10.53879/id.53.04.10455

8) Vu Dang Hoang et al, UV Spectrophotmetric Simultaneous Determination of Paracetamol and Ibuprofen in Combined Tablets by derivative and Wavelet Transforms, *The Scientific World Journa*l 2014,1-13.doi:10.1155/2014/313609.

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