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Development and Validation of a UV–Visible Spectrophotometric Method of Valacyclovir Hydrochloride in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract

The current study aimed to develop and validate simple and rapid UV-spectrophotometric method for the estimation of Valacyclovir Hydrochloride in bulk and pharmaceutical formulations as per ICH guidelines. A simple, fast, and accurate process for estimating valacyclovir hydrochloride in tablet and bulk form has been developed by UV spectroscopy in the current investigation. The solvent system employed in the proposed method were water, 0.25N hydrochloric acid, and 0.25N sodium hydroxide. Valacyclovir Hydrochloride found a good linear relationship over the concentration range 4-24 µg/ml in water, and 5 -30 µg/ml 0.25NHCl, and 0.25N NaOH respectively. The drug follows the linearity with a regression value of 0.9941, 0.9924, and 09935 in the respective solvents. The current investigation was also applied to tablet and % amount of drug which was estimated between 99.44% to 101.67% indicate the satisfaction level with the label claim. The recovery at 80%, 100%, and 120 level was measured in the range of 99.5 to 100%. Lessening of % RSD are revealing of the good accuracy and reproducibility of the method. It is clear from this study that the proposed methods for valacyclovir estimation in pharmaceutical preparation are quick, efficient, and specific. They may also be applied in routine analysis for the quantification of the drug in a dosage form. The current method was found simple and rapid tool for routine investigation of Valacyclovir Hydrochloride in the bulk and in the pharmaceutical dosage form.

Keywords: Antiviral, Acyclovir, Validation, Valacyclovir, UV

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Introduction

Valacyclovir is chemically L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9h-purin-9-yl) methoxy] ethyl ester, which is available as a pro-drug of acyclovir. The poor hydrophilicity and lipophilicity of acyclovir exhibit limited and variable oral bioavailability that reduces as the dose increases. Valacyclovir is subsequently treated by host thymidine kinase enzymes into acyclovir and valine, a naturally occurring amino acid [1]. Conversely, Valacyclovir possess 3 to 5 times more oral bioavailability than acyclovir. It is rapidly hydrolysed to acyclovir after oral administration due to its active transport by the human intestinal peptide transporter (PEPT1). It has proven antiviral potential against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV), both in vitro and in vivo. Both Valacyclovir and acyclovir are not metabolised by cytochrome P450 enzymes [2].

Bhavya Sri K et. al., 2025 [3] developed and validated an RP-HPLC method and a chromogenic UV–visible spectrophotometric method for the valacyclovir. The RP-HPLC method attained fast separation with a retention time of 3.177 min, displayed linearity over 10–60 µg/mL indicate the sensitive, accurate, precise, and specific approach. Beside that UV–visible method was based on the production of a green chromogen with MBTH in the presence of ferric ammonium sulfate at 618 nm. It was found linearity over 10–550 µg/mL, considered to human serum samples using a protein precipitation technique for bioanalytical applications. It was concluding that both approaches were suitable for routine pharmaceutical quality control and bioanalysis [3]

Chalikwar SS, Moravakar KK, and Bhairav BA et al., [4] produced UV spectrophotometric and HPLC methods for the quantitative estimation of valacyclovir hydrochloride in bulk drug and tablet dosage forms according to ICH Q2(R1) guidelines. In the UV spectrophotometric method, methanol and potassium dihydrogen phosphate (KH_2PO_4) buffer was employed at 254 nm. The method showed good linearity over the concentration range of 9–45 µg/mL, with a regression coefficient of 0.997. In HPLC method, Cosmosil C18 column employed with a mobile phase of methanol and 10 mM KH_2PO_4 buffer at a flow rate of 1.0 mL/min at 254 nm. Valacyclovir revealed a retention time of approximately 5.03

minutes, with an assay value of 99.56%. It demonstrating outstanding linearity over the concentration range of 10–50 µg/mL with a regression coefficient of 0.998 [4].

Morgan EM et al., [5] studied the different validation method including colorimetric method, the RP-HPLC and TLC-densitometric methods according to ICH guidelines. The colorimetric approach was found most simple, fast, economical, and highly sensitive, however the chromatographic techniques disclosed selectivity and sensitivity for the separation the drug from its degradation products in pharmaceutical formulations [5]. Therefore, in the current study is aimed to develop simple and rapid UV-spectrophotometric method for estimation of the Valacyclovir.

Materials and Methods

Valcivir 500 mg, manufactured by Cipla Pharma Ltd (tablet), was obtained from the local market of Dehradun, used as a sample. Valacyclovir HCl tablets comprising 500 mg Valacyclovir HCL, and the inactive ingredient used in the drug matrix. All chemicals and reagents employed were of analytical grade throughout the study and purchased from Marona Scientific, Dehradun, India. UV-Visible double beam spectrophotometer, Lab India, Model No.- UV 3200 was employed for the experiment.

A stock solution of 1000 µg /mL was prepared by dissolving accurately weighed Valacyclovir HCl reference standard (≥ 99.0% purity) individually in three solvent systems including water, 0.25 N hydrochloric acid (HCl), and 0.25 N sodium hydroxide (NaOH). Working standard solutions (2-30 µg/mL) were prepared by serial dilution from each stock by respective solvents. All solutions were scanned over the 200–400 nm range on a double-beam UV-Visible spectrophotometer using 1-cm quartz cuvettes. Each solvent showed a distinct absorption maximum (λ_{max}) arising from the n→π* transition of the guanine purine chromophore in the acyclovir moiety. Dilute hydrochloric acid is used as a diluent to simulate the acidic environment of gastric fluid (pH ~1.2–2.0), making it pharmacopoeia-relevant for drugs that are soluble or stable under acidic conditions. Sodium hydroxide solution is used as a diluent to simulate the alkaline environment of intestinal fluid (pH ~7.4–8.0) and is particularly suitable for drugs that are acidic in nature including NSAIDs, barbiturates, which ionise and dissolve readily under alkaline conditions. HCl and NaOH is a strong monoprotic acid and base; hence, its normality equals its molarity. An acidic medium also enhances the solubility of basic drugs through protonation and prevents their precipitation. An alkaline medium prevents degradation of acid-labile drugs and enhances absorbance in some chromophore systems through ionisation.

Selection of wavelength

50 mg of Valacyclovir HCl was dissolved separately in different solvents to prepare 4, 8, 12, 16, 20, 24, and 28 µg/ml concentration solutions. A stock solution of 100 µg/mL was prepared by dissolving accurately weighed 10 mg of Valacyclovir Hydrochloride in respective solvents up to 100 mL. From this stock solution, produced the concentration of 10 µg/ml which displayed λ_{max} at 252 nm, 256 nm, and 255 nm for water, 0.25 N HCl, and 0.25 N NaOH, respectively [6, 7].

Method Validation

The current method was validated in terms of linearity, accuracy, precision, robustness and specificity [8, 9].

Linearity study

For the linear study, three solvents were used to develop calibration curve in 0.25N HCl, 0.25N NaOH. 0.25 N NaOH. Calibration curves obeyed Beer-Lambert's Law in the different solvents. **Figure 1**.

Accuracy

The accuracy of the current method was evaluated by recovery studies at three different levels, i.e., 80%, 100%, 120%. The recovery studies were carried out by adding the known amount of standard solution of Valacyclovir HCl to pre-analysed solutions. The resulting solutions were then re-analysed by the proposed methods.

Precision

Precision of the current method was studied as intra-day and inter-day. Intra-day study and inter-day precision were performed by analysing, at different concentrations of drug, three times on the same day and for three days in a week.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were used to measure the sensitivity of the current methods were estimated in different solvents.

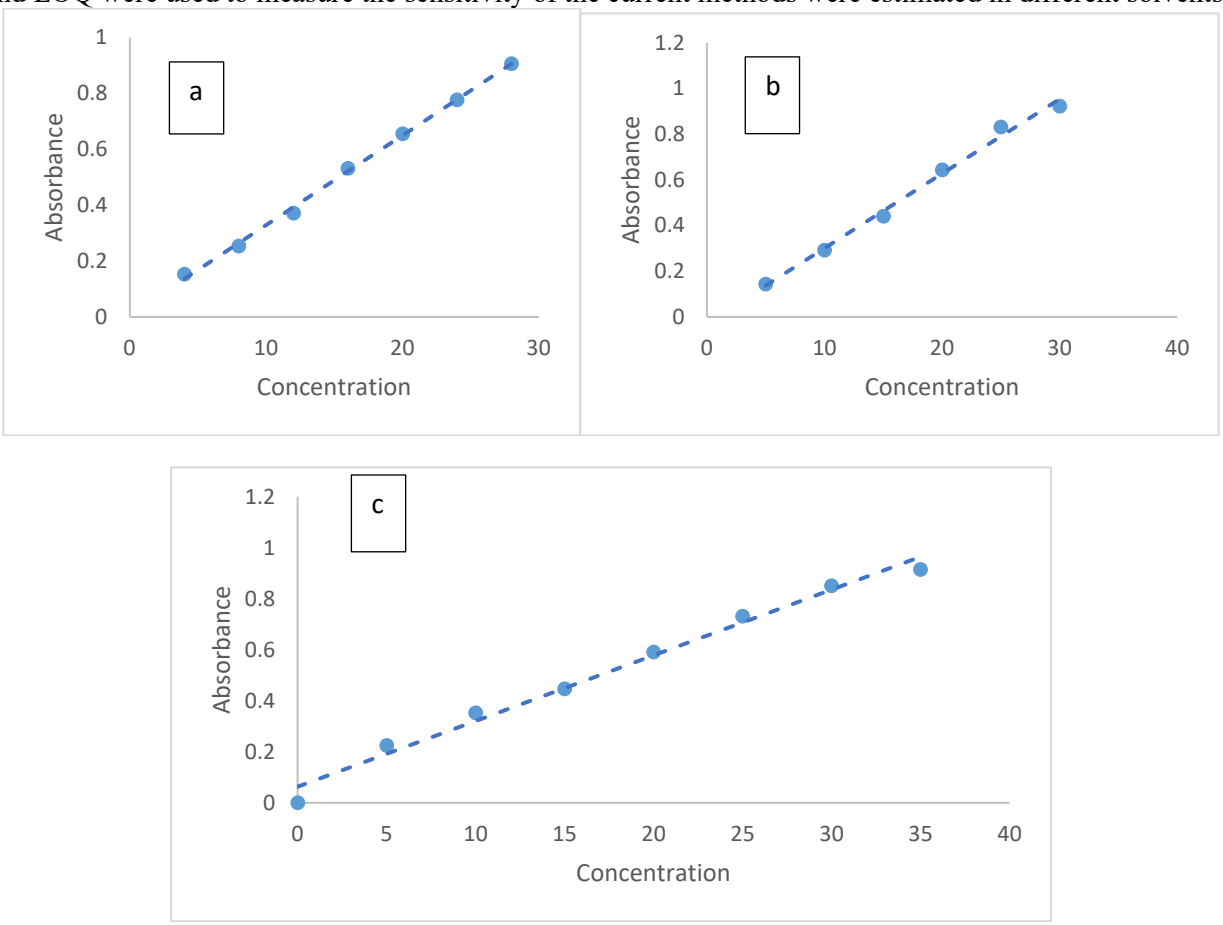

Figure 1 Calibration curve of Valacyclovir Hydrochloride: a) In water at 252 nm, b) 0.25N HCl at 256 nm, c) 0.25N NaOH at 255 nm

Specificity

The specificity of the current UV spectrophotometric method was evaluated by analysing the pure drug solution, control, and sample solution separately. The UV absorption spectra of all three solutions were recorded in the range of 200–400 nm. The control solution was prepared by dissolving a mixture of common tablet excipients including microcrystalline cellulose, magnesium stearate, povidone, and other fillers as claimed in Valcivir 500 mg. The absorbance of the control solution was measured at 256 nm and found to be negligible, indicating no interference from tablet excipients at the analytical wavelength. The UV spectrum of the sample solution was superimposed on that of the standard solution, and both showed identical λ_{max} at 256 nm with similar spectral profiles. These results confirm that the proposed method is specific for the determination of Valacyclovir HCl in the presence of common pharmaceutical excipients.

Robustness

Robustness evaluates the reliability of an analysis with respect to deliberate variations in method parameters. Small deliberate changes were introduced in the analytical wavelength (±1 nm from λ_{max}).

Determination of Valacyclovir Hydrochloride in bulk

Accurately weighed 10 mg of Valacyclovir HCl was transferred into a 100 mL volumetric flask, dissolved in 20 mL of distilled water, and the volume was made up to the mark with distilled water to obtain a stock solution of 100 µg/mL. From this stock solution, 0.8 mL was pipetted into a 10 mL volumetric flask and the volume was adjusted to the mark with distilled water to obtain a working concentration of 8 µg/mL. Distilled water was used as a blank. The solution was scanned on a UV-Vis spectrophotometer over 200–400 nm, and absorbance was recorded at 252 nm. The drug concentration was calculated using the linear regression equation obtained from the calibration curve.

Proposed method for pharmaceutical formulation

Twenty tablets of Valcivir 500 mg were weighed, and the average weight was calculated. Tablet powder equivalent to 10 mg of Valacyclovir was accurately weighed, transferred to a 100 mL volumetric flask, dissolved in distilled water, sonicated for 10 minutes, filtered, and made up to the mark to obtain a stock solution of 100 µg/mL. From this, 2 mL was pipetted into a 10 mL volumetric flask and the volume was adjusted to the mark with distilled water to obtain a working concentration of 20 µg/mL. The absorbance was measured at 252 nm against a distilled water blank, and the concentration was determined from the linear regression equation.

Result and Discussion

The solution was scanned on a UV-Vis spectrophotometer over the range 200–400 nm. Valacyclovir Hydrochloride exhibited λ_{max} at 252 nm in water, 256 nm in 0.25 N HCl, and 255 nm in 0.25 N NaOH. Based on these observations, distilled water was selected as the diluent and 252 nm was selected as the wavelength of maximum absorbance (λ_{max}) for further analysis **Figure 2**.

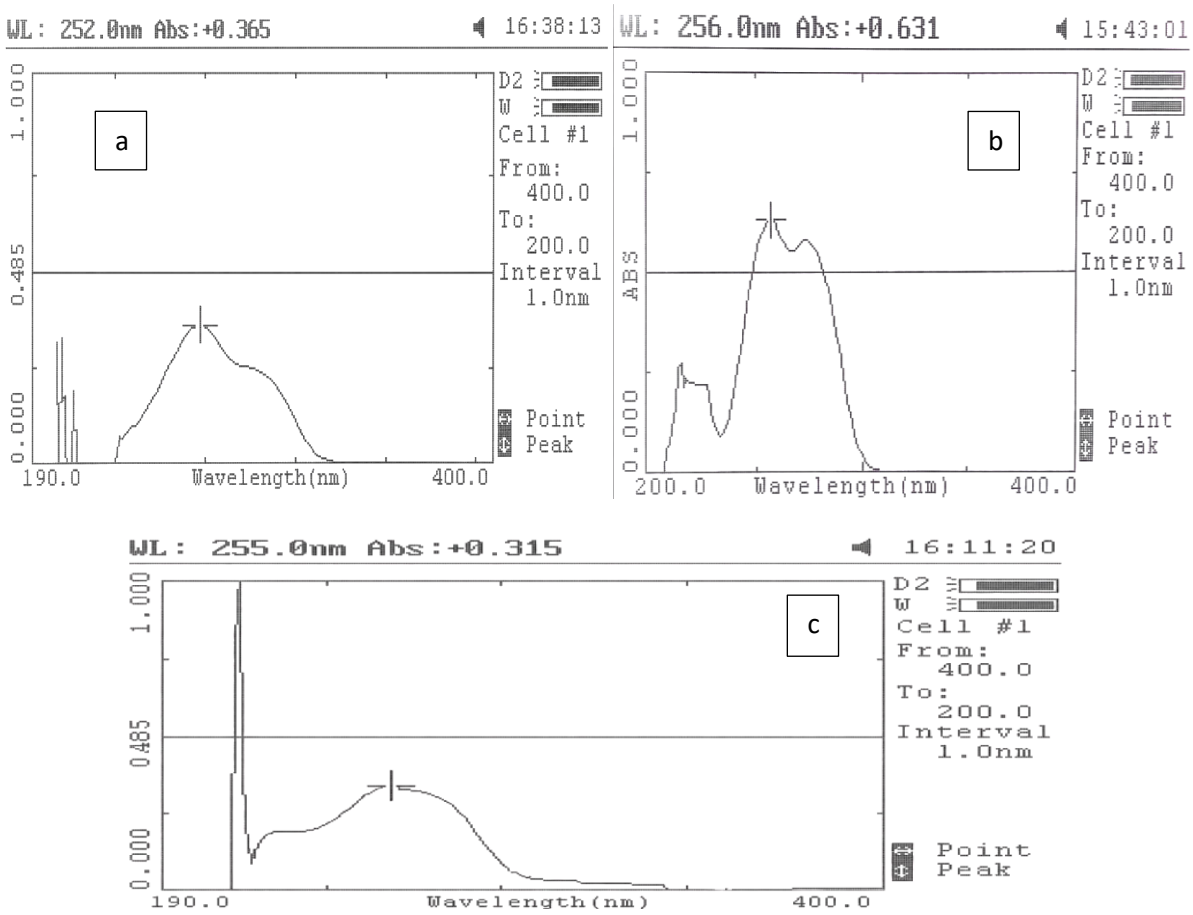

Figure 2 λ_{max} of Valacyclovir Hydrochloride in a) water, b) 0.25N HCl, c) 0.25N NaOH

Linearity study

Table 1 is the depiction of the linear regression data for the calibration curves showed a good linear relationship over the concentration range 4-28 µg/ml in water and 5 -30 µg/ml in 0.25N HCl and 5-35 µg/ml in 0.25N NaOH respectively. The linear regression equation was found to be $y = 0.0321x + 0.0082$ $R^2 = 0.9941$ for water, $y = 0.0326x - 0.0254$ $R^2 = 0.9924$ for 0.25N HCl, and $y = 0.0239x + 0.1084$ $R^2 = 0.9935$ for 0.25N NaOH. These findings are comparable to previously reported spectrophotometric methods for Valacyclovir Hydrochloride.

Accuracy

Results of recovery studies are reported in **Table 2**, which showed that the % amount found was between 98.54% and 99.98% with % RSD < 2.

Accuracy studies performed by the standard addition method at 80%, 100%, and 120% levels showed percentage

recoveries ranging from 99.50% to 100.00% with %RSD values below 2.0. The recovery values close to 100% indicate that the method is highly accurate and free from interference arising from excipients or solvent systems.

Precision

The precision of the developed method was expressed in terms of % Relative Standard Deviation (% RSD) or coefficient of variance (CV). The % RSD values found to be less than 2, that indicate the reproducibility of the assay **Table 3** and **Table 4**.

Precision studies, including intra-day and inter-day analyses, yielded %CV values below 3%, demonstrating good repeatability and intermediate precision of the developed method.

Table 1 Linearity study of Valacyclovir HCl in different solvents

Water		0.25N HCl		0.25N NaOH	
Conc.	**Absorbance**	**Conc.**	**Absorbance**	**Conc.**	**Absorbance**
4	0.153	5	0.144	5	0.225
8	0.254	10	0.292	10	0.352
12	0.372	15	0.441	15	0.446
16	0.532	20	0.643	20	0.591
20	0.656	25	0.831	25	0.731
24	0.778	30	0.922	30	0.850

Table 2 Summarization of accuracy data

Pre-analysed sample solution (µg/mL)	**Amount added (µg/mL) n=3**			**Amount recovered (µg/mL) n=3**			**% Recovery**			**% RSD**		
20	**Water**	**0.25N HCl**	**0.25N NaOH**	**Water**	**0.25N HCl**	**0.25N NaOH**	**Water**	**0.25N HCl**	**0.25N NaOH**	**Water**	**0.25N HCl**	**0.25N NaOH**
	8	8	7.98	7.96	7.98	7.96	99.5	99.75	99.75	1.36	1.58	1.56
	10	9.98	10	9.98	9.96	10	99.8	99.8	100	1.41	1.46	1.48
	12	11.99	12	11.97	11.82	11.97	99.75	99.58	99.75	1.31	1.33	1.29

Table 3 Intra-day Precision of Valacyclovir HCl in different solvent

Drug	**Conc. (µg/ml)**	**Abs Mean ± S.D. (n=3)**	**% CV**	**Wavelength at which estimation performed**
VAL in water	8	0.251±0.007	2.59	
	12	0.370±0.009	2.55	252 nm
	16	0.529±0.006	1.080	
VAL in 0.25N HCl	10	0.292±0.005	1.570	
	20	0.646±0.003	0.390	255 nm
	30	0.924±0.007	0.700	
VAL in 0.25N NaOH	15	0.449±0.004	0.890	
	20	0.586±0.006	1.040	256 nm
	25	0.729±0.003	0.350	

LOD and LOQ

Following equation is used to calculate The LOQ and LOD. LOD = 3.3 × N/B and LOQ = 10 × N/B, where 'N' is the standard deviation of the results (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve **Table 5**.

The sensitivity of the method was confirmed through the determination of LOD and LOQ values, which were found to be sufficiently low in all solvent systems. These results suggest that the proposed method can detect and quantify low concentrations of Valacyclovir Hydrochloride with acceptable accuracy and precision.

Specificity

Table 6 represents that proposed method is specific for the determination of Valacyclovir HCl in the presence of common pharmaceutical excipients in different solvents.

Specificity studies revealed negligible interference from commonly used tablet excipients. The UV spectra of the standard and sample solutions showed similar spectral characteristics with identical absorption maxima, confirming the ability of the method to selectively quantify Valacyclovir Hydrochloride in the presence of formulation components.

Table 4 Inter-day Precision of Valacyclovir Hydrochloride in different solvent

Conc. (µg/mL)	1st Day			2nd Day			3rd Day		
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Valacyclovir HCl in water									
8	0.251	0.007	2.59	0.290	0.001	0.34	0.302	0.005	1.72
12	0.370	0.009	2.550	0.451	0.012	2.57	0.455	0.006	1.400
16	0.529	0.006	1.080	0.578	0.008	1.35	0.585	0.005	0.900
Valacyclovir HCl in 0.25N HCl									
10	0.292	0.005	1.570	0.287	0.005	1.61	0.293	0.007	0.700
20	0.646	0.003	0.390	0.651	0.010	1.54	0.643	0.005	0.740
30	0.924	0.007	0.700	0.927	0.004	0.45	0.930	0.002	0.220
Valacyclovir HCl in 0.25N NaOH									
15	0.449	0.004	0.890	0.444	0.007	1.50	0.447	0.004	0.900
20	0.586	0.006	1.040	0.587	0.004	0.74	0.584	0.005	0.860
25	0.729	0.003	0.350	0.726	0.008	1.04	0.726	0.005	0.730

Table 5 Summarization of LOD and LOQ in different solvents of Valacyclovir HCl

Drug	LOQ (µg/ml)			LOD (µg/ml)		
	1st Day	2nd Day	3rd Day	1st Day	2nd Day	3rd Day
Valacyclovir HCl in water	6.852	6.53	4.96	2.260	2.16	1.644
Valacyclovir HCl in 0.25N HCl	4.600	3.07	4.293	1.517	1.92	1.416
Valacyclovir HCl in 0.25N NaOH	5.348	7.95	5.857	1.794	2.62	1.93

Table 6 Summarization of specificity of Valacyclovir HCl in different solvents

Valacyclovir HCl	Conc. (µg/ml)	Before addition of excipients		After addition of excipients		% Interference
		Abs.	Conc.	Abs.	Conc.	
Water	8	0.253	8.3	0.255	8.4	0.79
	12	0.363	11.8	0.372	12.1	2.48
	16	0.531	16	0.544	16.3	2.45
0.25 N HCL	10	0.299	9.9	0.308	10.2	3.01
	20	0.652	20	0.657	20.1	0.77
	30	0.928	30.1	0.934	30.3	0.65
0.25N NaOH	15	0.443	15.1	0.452	15.4	2.03
	20	0.587	19.8	0.592	20.3	0.85
	25	0.732	25.1	0.736	25.2	0.55

Robustness

The absorbance values at all tested wavelengths including 251 nm,252 nm, and 253nm were found constant, with lessening of RSD%, confirming the analytical method is robust. **Table 7**.

Furthermore, robustness studies demonstrated that minor deliberate variations in wavelength (±1 nm) did not significantly affect the absorbance values, as reflected by the low %RSD values obtained. This confirms the reliability and ruggedness of the analytical procedure under normal laboratory conditions.

Determination of Valacyclovir hydrochloride in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.44% to 101.67%. Application of the validated method to the marketed formulation (Valcivir 500 mg tablets) resulted in an assay value of 100.4% of the labelled claim with low variability (%RSD = 0.70), indicating excellent agreement

with the declared content. Recovery studies conducted on the formulation further supported the suitability of the method, with recovery values ranging from 98.50% to 102.22% in different media, which fall within acceptable analytical limits.

Table 7 Summarization of specificity of Valacyclovir HCl in different solvents at different wavelength

	Water			0.25N HCl			0.25NaOH		
S. No.	**251 nm**	**252 nm**	**253 nm**	**255 nm**	**256 nm**	**257 nm**	**254 nm**	**255 nm**	**256 nm**
1	0.528	0.532	0.536	0.437	0.441	0.445	0.587	0.591	0.595
2	0.528	0.532	0.535	0.437	0.441	0.444	0.587	0.591	0.594
3	0.529	0.533	0.536	0.438	0.442	0.445	0.588	0.592	0.595
4	0.529	0.533	0.536	0.438	0.442	0.445	0.588	0.592	0.595
5	0.529	0.533	0.536	0.438	0.442	0.445	0.588	0.592	0.595
6	0.53	0.534	0.537	0.439	0.443	0.446	0.589	0.593	0.596
Mean	0.5288	0.5328	0.536	0.4378	0.4418	0.445	0.5878	0.5918	0.595
SD	0.0008	0.0008	0.0007	0.0008	0.0008	0.0007	0.0008	0.0008	0.0007
% RSD	0.151	0.15	0.131	0.183	0.181	0.157	0.136	0.135	0.118

Proposed method for pharmaceutical formulation

The assay of the marketed tablet formulation Valcivir, having a label claim of 500 mg, was carried out successfully. The amount of drug found was 502 mg per tablet, corresponding to 100.4% of the label claim. The percentage %RSD was found to be 0.70, indicating good precision and reproducibility of the analytical method. The results confirm that the formulation complies with the acceptable pharmaceutical standards for drug content uniformity and accuracy. The table exhibits the recovery data for the opted method for pharmaceutical formulation.

The recovery studies of Valacyclovir HCl were performed in water, 0.25 N HCl, and 0.25 N NaOH at three different concentration levels (80%, 100%, and 120%) to evaluate the accuracy of the analytical method. In water, the percentage recovery values ranged from 98.89% to 101.36%, indicating satisfactory recovery of the drug. In 0.25 N HCl, the recovery values were found between 98.50% and 101.11%, while in 0.25 N NaOH, the recovery ranged from 98.50% to 102.22%. The results obtained in all media were within acceptable limits, demonstrating that the proposed method is accurate, reliable, and free from interference by excipients or degradation products [10].

Application of the validated method to the marketed formulation (Valcivir 500 mg tablets) resulted in an assay value of 100.4% of the labelled claim with low variability (%RSD = 0.70), indicating excellent agreement with the declared content. Recovery studies conducted on the formulation further supported the suitability of the method, with recovery values ranging from 98.50 % to 102.22 % in different media, which fall within acceptable analytical limits.

Water, 0.25 N hydrochloric acid, and sodium hydroxide is easily accessible to demonstrate that the analytical method is reliable, accurate, and reproducible under different pH conditions as well as expand the applicability of the analytical method for different pharmaceutical and research purposes across a wide range of experimental conditions. The solubility, ionization, and absorption characteristics of many pharmaceutical compounds can change with pH, which may influence their UV spectra and analytical performance. Current approaches offer versatility, economic, broader applicability, stronger scientific evidence of its reliability and robustness. Overall, validating the method in different media enhances its flexibility and usefulness throughout the pharmaceutical product lifecycle. Acidic and alkaline media are widely used for the dissolution studies and stability studies.

Conclusion

The current method of UV spectrophotometric by using different solvents satisfies the validation requirements endorsed by ICH guidelines with respect to linearity, accuracy, precision, specificity, robustness, and sensitivity. The key note of the investigation were simplicity of sample preparation, economic, and short time management appropriate for routine quality control analysis of Valacyclovir HCl in bulk drug substances and pharmaceutical dosage forms.

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