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**RESEARCH ARTICLE**

# Stability-Indicating RP-HPLC Method for the Determination of Valaciclovir Hydrochloride in Bulk and Pharmaceutical Dosage Forms

DILLIP KUMAR SAHOO[*1] and PRAFULLA KUMAR SAHU[2]

[1]Department of Pharmaceutical Analysis, Avanthi Institute of Pharmaceutical Sciences, Cherukupally, Bhogapuram, Vizianagaram, Andhra Pradesh-531162, India

[2]Department of Pharmaceutical Analysis, Raghu College of Pharmacy, Dakamarri, Bheemunipatnam, Visakhapatnam, Andhra Pradesh-531162, India

*sahoo4@rediffmail.com*

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**Abstract:** A stability indicating reverse-phase HPLC method was developed for the determination of valaciclovir hydrochloride present in bulk and pharmaceutical dosage forms. The quantification was carried out using hypersil, ODS $C_{18}$ (250×4.6 mm i.d., packed with 5 µ particle size) in an isocratic mode column with mobile phase comprising acetonitrile: phosphate buffer (pH- 3.6) in the ratio of 50:50 (%v/v). The flow rate was at 0.8 mL/min and detection was carried out at 252 nm. The retention time was 2.850 min for valaciclovir hydrochloride and the method produced linear response in the concentration range of 0.5 to 200 µg/mL ($R^2$~0.9999). The recovery studies were also carried out and percent relative standard deviation from reproducibility was below 2%. The limit of detection and limit of quantification for this method were 0.110 µg/mL and 0.363 µg/mL, respectively. The proposed method was statistically evaluated and can be applied for routine quality control analysis of valaciclovir hydrochloride in Pharmaceutical dosage form.

**Keywords:** Valaciclovir, Stability indicating, RPHPLC

## Introduction

Valaciclovir or valaciclovir is an antiviral drug used in the management of herpes simplex and herpes zoster (shingles). It is a prodrug, being converted *in vivo* to aciclovir. Many analytical methods are developed in order to determine valaciclovir in bulk and tablet dosage forms. Spectrophotometric[1-2] methods were developed for the determination of valaciclovir in bulk and pharmaceutical dosage forms. RPHPLC methods were reported for estimation of valaciclovir in presence of degradation products[3], to obtain degradation kinetics data[4] in aqueous solution and *L*-valine, ester with 9-[(2-hydroxyethoxy) methyl] guanine hydrochloride in tablets[5], in human serum[6] to carry out drug dissolution studies. Gladys E Graneroa *et al.,*[7] has described a method for the stability of valaciclovir and implications for its oral bioavailability. A.S Jadhav *et al*.,[4] developed an enantioselective

chiral high performance liquid chromatographic method for the enantiomeric resolution of valaciclovir. Two liquid chromatography mass spectrometry (LC-MS) methods are reported for the quantification of valaciclovir and its metabolite[8] and simultaneous determination of valaciclovir and acyclovir in human plasma. Studies on electrooxidation and its square-wave and differential pulse voltammetric determination in pharmaceuticals and human biological fluids were reported by Bengi Uslua *et al.,*[9]. A human valaciclovirase biphenyl hydrolase-like protein was identified as valaciclovir hydrolase by Insook Kim *et al.,*[10] which may be concluded as an important enzyme activating valaciclovir and valganciclovir in humans and an important new target for prodrug design.

The objective of this study is to develop and validate a simple and cost effective stability indicating method for rapid estimation of valaciclovir in presence of its stress degradation related impurities. Hence the proposed method can be useful as a rapid analytical technique for the degradation kinetics and to establish the degradation pathways.

## Experimental

HPLC- Shimadzu Class-VP series with Class-VP Software, sonicator- Sharp Analyticals, rotary shaker- Vibramax, analytical balance- Metler Toledo, pipettes- Thermo Electron.

### Preparation of solutions

#### *Preparation of mobile phase*

Acetonitrile and phosphate buffer (pH- 3.6) were properly mixed in the ratio of 50:50. For the preparation of phosphate buffer solution (pH-3.6), 0.9 g of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate were weighed accurately, mixed and volume made with double distilled water (1000 mL).

#### *Preparation of standard drug solutions*

Stock solution of valaciclovir (1 mg/mL) was prepared by dissolving 25 mg of valaciclovir in 25 mL of volumetric flask containing 10 mL of acetonitrile and 10 mL of phosphate buffer (pH-3.6). The solution was sonicated for about 10 min and then made up to volume with mobile phase. Daily working standard solutions of valaciclovir was prepared by suitable dilution of the stock solution with appropriate mobile phase. Working standard solutions of valaciclovir were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000 µg/mL and the volume was made up to 10 mL with mobile phase.

### Method development

#### *Method optimization*

To develop a suitable and robust HPLC method for the determination of valaciclovir, different mobile phase compositions in different ratios were used at different flow rates.

#### *Method validation*

The validation parameters like linearity, sensitivity, accuracy, precision, robustness and specificity of the assay and the recovery were studied according to the US Food and drug administration (FDA) guidance for the validation of analytical methods.

Calibration curves were prepared by assaying standard sample solutions ranging from 1 to 200 µg/mL. The linearity of each method matched calibration curve was determined by plotting the peak area ($y$) *versus* the concentration ($x$) of valaciclovir.

*Assay of formulation*

Twenty tablets were weighed and finely powdered. An accurately weighed sample of powdered tablets equivalent to 25 mg of valaciclovir was extracted with acetonitrile and phosphate buffer (pH-3.6) using ultra sonicator in a 25 mL volumetric flask followed by filtration through 0.45 µm filter paper. The solution obtained was further diluted with the mobile phase so as to get the desired concentrations. The amount of drug present in pharmaceutical formulation was calculated by the following formula.

$$\% \text{ Assay} = \frac{\text{Sample area x standard weight x Average weight of 20 tablets}}{\text{Standard area x Sample weight x Label claim}}$$

The limit of detection (LOD) and limit of quantitation (LOQ) were determined on the basis of response and slope of the regression equation. The precision of the method was ascertained separately from the areas under the curve obtained by actual determination of eight replicates of a fixed amount of drug and the percent relative standard deviations were calculated. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples of valciclovir within the linearity ranges were taken and added to the pre-analyzed formulation of concentration 10 µg/mL. From that percentage recovery values were calculated.

To study the robustness of the method, the test solutions were injected with deliberate variations in method parameters like flow rate, temperature, pH and mobile phase composition. For the ruggedness study, the prepared test solution as per the test method was analyzed by different analysts on a different instrument using the same column.

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acidic, alkaline, oxidative and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

## Results and Discussion

*Chromatographic conditions*

The $\lambda_{max}$ of valaciclovir was found to be 252 nm. The mobile phase acetonitrile: phosphate buffer (pH-3.6) of 50:50%v/v at a flow rate of 0.8 mL/ min gave peaks with good resolution for valaciclovir are eluted at retention time around 2.850 min and with symmetric peak shape.

*Method validation*

*Linearity*

When a series of dilutions were analyzed, the concentration range of 1 to 200 µg/mL was found to give a straight line. The linearity study is given in Figure 1 and the typical chromatogram of the pure drug is given in Figure 2.

*Assay of formulation*

The amount of drug present in pharmaceutical formulation was calculated to be 100.275% with SD of 0.205 and %RSD of 0.204. A typical chromatogram of valaciclovir in formulation was shown in Figure 3 and the assay results are given in Table 1.

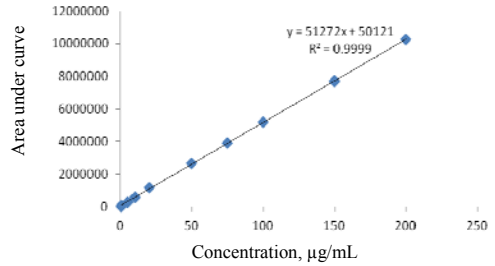


**Figure 1.** Linearity graph of valaciclovir

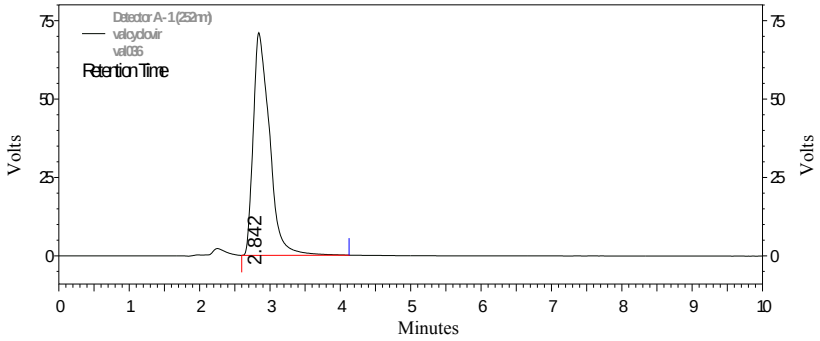


**Figure 2.** A Typical chromatogram of valaciclovir in pure drug (50 µg/mL)

**Table 1.** Assay of formulation

| Formulation | Labeled Amount, mg | Mean ±SD (amount recovered) | %Drug recovered | % RSD |
|---|---|---|---|---|
| Valcivir (tablets) | 500 | 501.377±1.025 | 100.275±0.205 | 0.204 |

**Each value is average of six determinations ± standard deviation*

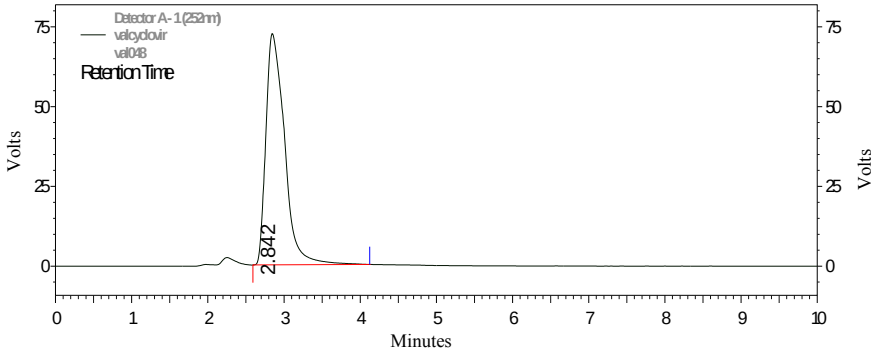


**Figure 3.** A Typical chromatogram of valaciclovir in formulation (50 µg/mL)

*Precision and accuracy*

The method was found to be precise and the SD and %RSD of the area under curves were calculated to be 10408.85 and 1.763 respectively and presented in the Table 2. The accuracy of the method was determined by recovery studies and percentage recovery values were calculated. The accuracy results were shown in Table 3.

**Table 2.** Precision study

| S. No. | Concentrations µg/mL | AUC | Statistical analysis |
|---|---|---|---|
| 1 | 10 | 582534 | |
| 2 | 10 | 601255 | |
| 3 | 10 | 596824 | Mean = 590218.5 |
| 4 | 10 | 572155 | |
| 5 | 10 | 582456 | SD = 10408.85 |
| 6 | 10 | 600345 | |
| 7 | 10 | 589325 | %RSD = 1.76356 |
| 8 | 10 | 596854 | |

**Table 3.** Accuracy study

| Sample | Concentration µg/mL | | %Recovery of pure drug | Statistical Analysis | |
|---|---|---|---|---|---|
| | Pure drug | Formulation | | | |
| S1 : 80 % | 8 | 10 | 98.381 | Mean | 98.1337 |
| S2 : 80 % | 8 | 10 | 97.56 | SD | 0.49838 |
| S3 : 80 % | 8 | 10 | 98.46 | % RSD | 0.50786 |
| S4 : 100 % | 10 | 10 | 101.56 | Mean | 101.4633 |
| S5 : 100 % | 10 | 10 | 101.89 | SD | 0.48232 |
| S6 : 100 % | 10 | 10 | 100.94 | % RSD | 0.475365 |
| S7 : 120 % | 12 | 10 | 99.61 | Mean | 99.2533 |
| S8 : 120 % | 12 | 10 | 99.28 | SD | 0.37072 |
| S9 : 120 % | 12 | 10 | 98.87 | % RSD | 0.373509 |

*Limit of detection and quantitation*

The parameters LOD and LOQ for this method were found to be 0.110 µg/mL and 0.363 µg/mL respectively.

*Robustness*

To study the robustness of the method, the test solutions were injected with deliberate variations in method parameters like flow rate, temperature, pH and mobile phase composition. The reliability of the method is shown by the robustness study result given in Table 4.

**Table 4.** Robustness study (10 µg/mL)

| Parameters (n=6) | Variables | Statistical analysis | | Statistical analysis | |
|---|---|---|---|---|---|
| | | RT Mean ±SD | %RSD | Peak area Mean ±SD | %RSD |
| Flow rate (mL/min) | 0.7 | 2.92±0.05 | 1.70 | 574145±17751.79 | 3.09 |
| | 0.8 | 2.85±0.05 | 1.75 | 582456±10408.85 | 1.78 |
| | 0.9 | 2.63±0.09 | 3.42 | 569450±15529.48 | 2.72 |
| Mobile phase Composition (Buffer : ACN) | 55 : 45 | 2.93±0.10 | 3.41 | 582659±13499.23 | 2.31 |
| | 50:50 | 2.85±0.05 | 1.75 | 582456±10408.85 | 1.78 |
| | 45 : 55 | 2.75±0.09 | 3.27 | 583254±18063.72 | 3.09 |
| Temperature ºC | 26 | 2.88±0.09 | 3.12 | 572425±11496.93 | 2.00 |
| | 28 | 2.85±0.05 | 1.75 | 582456±10408.85 | 1.78 |
| | 30 | 2.76±0.04 | 1.44 | 583254±10373.34 | 1.77 |
| pH | 3.4 | 2.75±0.05 | 1.81 | 576382±12682.56 | 2.200 |
| | 3.6 | 2.85±0.05 | 1.75 | 582456±10408.85 | 1.78 |
| | 3.8 | 2.79±0.06 | 2.15 | 586467±16632.92 | 2.83 |

*Ruggedness*

The prepared test solution as per the test method was analyzed by different analysts on a different instrument using the same column. The ruggedness study result is given in Table 5.

**Table 5.** Ruggedness study

| Variables | RT mean ±SD | %RSD | Peak area mean ±SD | %RSD |
|---|---|---|---|---|
| Analyst-I | 2.75±0.05 | 1.81 | 576382±10282.56 | 1.20 |
| Analyst-II | 2.85±0.05 | 1.75 | 582456±10408.85 | 1.78 |

*Forced degradation studies*

About 10 mg of valaciclovir pure drug was accurately weighed and transferred to 10 mL volumetric flask which was further treated with different stress conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. The summary of results of stability indicating assay is given in Table 6. From the specificity study it was found that in all types of stress conditions the drug is degrading. Furthermore Valaciclovir is more susceptible to alkaline conditions.

**Table 6.** Results of stability indicating assay

| Conditions applied | Peak area | %Drug recovered | RT of analyte min | RT of major degradants min |
|---|---|---|---|---|
| Standard drug (SD) | 582534 | 100.0 | 2.842 | - |
| 10 µg of SD + 1 mL of 0.1 N HCl (1 h) | 573961 | 98.52833 | 2.842 | 1.967, 2.258 |
| 10 µg of SD + 1 mL of 0.1 N NaOH (1 h) | 47643 | 81.78578 | 2.842 | 1.992, 2.242 |
| 10 µg of SD + 1 mL of 3%v/v $H_20_2$ (1 h) | 572307 | 98.24439 | 2.842 | 1.950, 2.242 |
| 10 µg of SD solution under UV (8 h) | 544850 | 93.53102 | 2.842 | 1.967, 2.250 |

*Acidic degradation*

About 10 mg of valaciclovir pure drug was accurately weighed and transferred to 10 mL volumetric flask and 1 mL of 0.1 N HCL was added and kept aside for one h and made up to volume with mobile phase. Then from this 10 µg/mL solution was prepared and injected in HPLC system to obtain chromatograms (Figure 4).

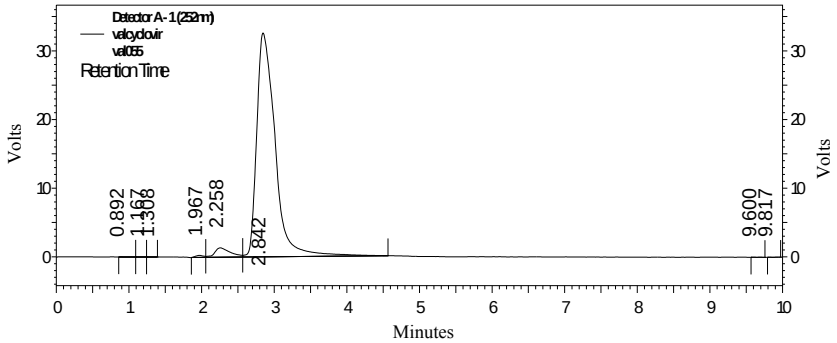


**Figure 4.** Chromatogram for acidic degradation

*Alkaline degradation*

About 10 mg of valaciclovir pure drug was accurately weighed and transferred to 10 mL volumetric flask and one mL of 0.1 N NaOH was added and kept aside for one hour and made up to volume with mobile phase. Then from this 10 µg/mL solution was prepared and injected in HPLC system to obtain chromatograms (Figure 5).

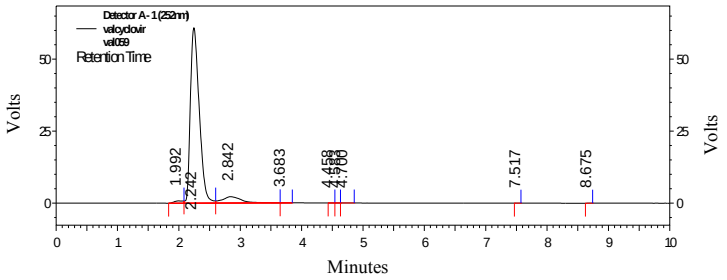


**Figure 5.** Chromatogram for alkaline degradation

*Oxidative degradation*

About 10 mg of valaciclovir pure drug was accurately weighed and transferred to 10 mL volumetric flask and one mL of 3%w/v of hydrogen peroxide ($H_2O_2$) was added and kept aside for two hrs and made up to volume with mobile phase. Then from this 10 µg/mL solution was prepared and injected in HPLC system to obtain chromatograms (Figure 6).

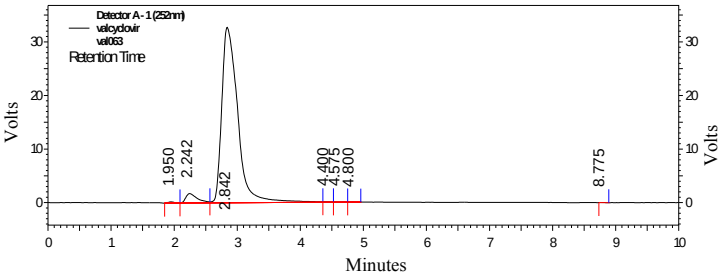


**Figure 6.** Chromatogram for oxidative degradation ($H_2O_2$)

*Photolysis*

About 10 mg of valaciclovir pure drug was accurately weighed and transferred to 10 mL volumetric flask and made up to volume with mobile phase and kept aside for 8 h under direct sunlight. Then from this 10 µg/mL solution was prepared and injected in HPLC system to obtain chromatograms (Figure 7).

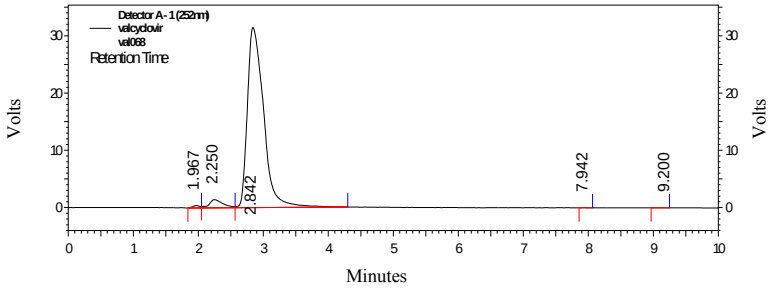


**Figure 7.** A Typical chromatogram for photolytic UV degradation

*System suitability testing*

System suitability test of the HPLC method gave good resolution ($R$=2.57), relative retention time ($\alpha$=2.2), column capacity ($K$=2.4) and tailing factor ($T$=1.024). The system suitability parameters are given in Table 7.

**Table 7.** System suitability

| Parameters | Obtained value | Reference value |
|---|---|---|
| Relative retention *(α)* | 2.2 | >1 |
| Tailing factor *(T)* | 1.024 | <1.5–2 or <2 |
| Capacity factor *(K')* | 2.4 | 1–10 acceptable |
| Theoretical plates *(N)* | 4369 | >2000 |
| Resolution *(R)* | 2.57 | R > 0.8 |

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