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# VALIDATED STABILITY-INDICATING RP-HPLC ASSAY METHOD FOR VALGANCICLOVIR HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM ACCORDING TO ICH GUIDELINES

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## ABSTRACT

**Objective:** A simple, sensitive, precise and accurate stability-indicating HPLC method has been developed and validated for determination of Valganciclovir hydrochloride in bulk drug and in pharmaceutical dosage form in the presence of degradation products. **Methods:** An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software. Mobile phase consists of mixture of 0.01M sodium dihydrogen phosphate buffer (A) and Acetonitrile (B) in the ratio (pH 5.0±0.1, ratio 60:40 v/v) at a flow rate of 1.0 mL /min. The linear regression analysis data for the calibration curve showed a good linear relationship with regression coefficient 0.9996. The detection was carried out at a wavelength of 254nm. **Results:** The linearity of the method were excellent over range 2-12µg/ml, the linear regression equation was Y = 118614x + 7332.9. The Valganciclovir hydrochloride was subjected to stress conditions of hydrolysis (acid, base), photolysis and thermal degradation. Degradation was observed for Valganciclovir hydrochloride in acid, base, heat and UV. The degradation products were well resolved from the main peak. The percentage recovery of Valganciclovir hydrochloride was from (98.0 to 102.0 %.) in the pharmaceutical dosage form. **Conclusion:** The results demonstrated that the method would have a great value when applied in quality control and stability studies. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, specificity and robustness according to the ICH guidelines. The forced degradation studies prove the stability indicating power of the method.

**KEYWORDS:** Valganciclovir hydrochloride, HPLC, 0.01M sodium dihydrogen phosphate buffer**:** Acetonitrile (60:40), ICH guidelines and forced degradation studies.

## INTRODUCTION

Valganciclovir hydrochloride is a hydrochloride salt form of Valganciclovir, a prodrug form of ganciclovir, a nucleoside analogue of 2'-deoxyguanosine, with antiviral activity. After the completion of phosphorylation, Valganciclovir is incorporated into DNA, resulting in the inhibition of viral DNA polymerase, and viral replication. Valganciclovir hydrochloride is an antiviral agent that is used to treat cytomegalovirus retinitis in patients with AIDS, and for the prevention of cytomegalovirus infections in organ transplant recipients who have received an organ from a CMV-positive donor. The Valganciclovir acts by slowing the growth of the CMV virus. It helps prevent the spread of infection to other areas of the body.[2]

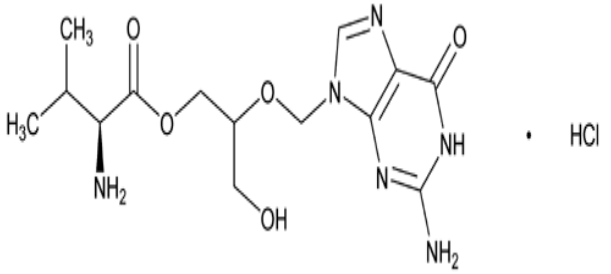


**Figure 1: Chemical structure of Valganciclovir hydrochloride.[1]**

Valganciclovir hydrochloride contains not less than 97% and not more than 102%. Hydrochloride is calculated based on the anhydrous and solvent free basis. Valganciclovir hydrochloride is available in the form of White to off crystalline powder. The crystals from water+isopropranol undergo phase changes at 142˚C. It is freely soluble in Water, Dimethyl sulfoxide, Methanol,

Acetonitrile and Acetic acid. Valganciclovir hydrochloride is available under the brand name of Valcyte, Cymeral, Rovalcyte and Darilin.[2-6]

Literature Survey revealed that the drug has been estimated by UV-Spectrophotometric,[11-13] RP-HPLC,[14-17] HPTLC,[18] and Liquid chromatographic method,[19-21] has been reported so far.

The present study describes a simple, precise and accurate analytical method for the estimation of Valganciclovir hydrochloride in bulk and pharmaceutical dosage forms. The above method was developed and validated according to the ICH guidelines.

## MATERIALS AND METHODS

**Material and reagents:** The Valganciclovir hydrochloride was obtained as a gift sample from the pharmaceutical industry and tablet Valganciclovir hydrochloride were obtained from Pharmacy store. Hydrochloric acid, sodium hydroxide pellets, dihydrogen phosphates and Acetonitrile from were obtained Bharthi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

### Instrumentation

Chromatographic separation was performed on a Shimadzu LC-20AT HPLC system comprising a variable wavelength programmable UV/ VIS detector SPD-20A (VP- series), Shimadzu LC-20AT (VP series) pump and Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software.

### Chromatographic conditions

**Table 1: HPLC method development parameters.**

| HPLC method development parameters | |
|---|---|
| Column | C18, 150 X 4.6 mm, 5µ |
| Flow rate | 1.0 mL / min |
| Wavelength | 254 nm |
| Column temperature | 30°C |
| Injection volume | 50 µL |
| Run time | 10 minutes |
| Diluents | Mobile phase |
| Elution | Isocratic |

### Preparation of solutions

### Mobile phase preparation

2.72gms of sodium dihydrogen phosphate and 2ml triethylamine in 1000ml water, Adjust pH 5.0±0.1 with dilute orthophosphoric acid.

Mobile phase: 0.01M sodium dihydrogen phosphate buffer: Acetonitrile (60:40)

The mobile phase was premixed and filtered through a 0.45µ nylon filter and degassed.

### Preparation of stock and standard solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of Valganciclovir hydrochloride was prepared by dissolving the drug in the diluents and diluting them to the desired concentration. Diluents were composed of Potassium dihydrogen ortho phosphate and Acetonitrile in the ratio (60: 40 v/v). Approximately 100 mg of Valganciclovir hydrochloride was accurately weighed, transferred in a 100 mL volumetric flask, add 30 mL of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent. And an appropriate concentration of sample was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

### Preparation of sample solution

Crush to powder 20 tablets of Valganciclovir hydrochloride weigh and transfer the tablet powder equal to 100 mg of Valganciclovir hydrochloride into 100 mL volumetric flask add 30 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter. And an appropriate concentration of sample (was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and preceded as said for the standard respectively.

### Assay

Diluted to 10 ml of standard stock solution, into 100 mL and make up to volume with diluent.

Repeat the same procedure for three preparations.

### System suitability requirements from stock and standard solutions

a) Tailing factor: NMT 2.0
b) Theoretical Plates: NLT 2000

### Procedure for forced degradation study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that plays an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies.

### Acidic degradation

5 mg drug were dissolved in the diluents A&B. Forcibly degrade the sample by using 0.1 N HCl at room temperature. Collect 10 mL of the sample after 48 hours.

**Alkaline degradation**
5 mg drug were dissolved in the diluents A&B. Forcibly degrade the the sample using 0.1N NaOH at room temperature. Collect 10 mL of the sample after 48 hours.

**Thermal degradation**
10 mg drug were forcibly degrading the sample exposed to heat under over 75˚C of temperature. The the working solutions was prepared using the diluents A&B. Collect the sample after 10 th day.

**Photo degradation**
10 mg of drug is exposed to the short wavelength (254 nm) UV light for 48 h. Then the working solution was prepared by using diluents A&B. Forceibly degrades the sample under UV.

**Method validation**
**Specificity**
Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix the specificity of the developed HPLC method for Valganciclovir hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Valganciclovir hydrochloride in bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions exposing it with acid (01N hydrochloric acid), alkali (0.1N NaOH), heat (75˚C) and UV light (254 nm wavelength) to evaluate the ability of the proposed method to separate AZaz from its degradation products. For light and heat study, the study period was 48 h whereas for acid and base 48 h. Peak purity test for Valganciclovir hydrochloride was by using UV/ Visible detector in stress samples.

**Precision**
Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of Valganciclovir hydrochloride test samples against reference standard; the percentage of RSD of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different HPLC systems and different days in the same laboratory the results were tabled in Table 3.

**Accuracy (recovery test)**
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (50 mg of Valganciclovir hydrochloride). The recovery samples were prepared in the before mentioned procedure, and then 10 mL of Valganciclovir hydrochloride solution were transferred into a 50 mL volumetric flask and diluted to volume with diluent B. Three samples were prepared for each recovery level. The Solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Valganciclovir hydrochloride ranged from 98.0 to 102.0%. The average recoveries of three levels of Valganciclovir hydrochloride were found to be 99.86%. the results are depicted in the table 4.

**Linearity**
The linearity of the response of the drug was verified at six concentration levels, ranging from 25 to 150% of the targeted level**.** Concentration standard solutions containing 2-12µg/ ml of Valganciclovir hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area versus the concentration data and were treated by least-squares linear regression analysis. The equation of the calibration curve for Valganciclovir hydrochloride obtained Y = 118614x + 7332.9, the Calibration graphs were found to be linear in the aforementioned concentrations. The correlation coefficient of determination is 0.9996. The linearity calibration curve was showed in figure-2. The results were tabulated in table-5. The sample Chromatogram showed in Figure-4.

The limit of detection (LOD) and limit of quantification (LOQ) were determined by calibration curve method. Specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. The LOD and LOQ for Valganciclovir hydrochloride in LC were 0.076 and 0.232respectively. The LOD and LOQ were calculated using the following formula LOD=3.3SD/b, LOQ=10SD/b. where SD is the standard deviation of the calibration curve and b is the slope of the calibration curve. Precision at limit of quantification and limit of detection was checked by analyzing six test solutions prepared at LOQ and LOD levels and calculating the percentage RSD of area.

**Robustness**
To determine the robustness of the developed method experimental conditions were purposely altered and the resolution between Valganciclovir hydrochloride and acid degradation products were evaluated. The flow rate of the mobile phase was 1.0 mL min± 0.1 ml, and temp variation ± 5$^{o}$C to study the effect of flow rate on the resolution. The effect of percent organic strength on resolution was studied by varying. Acetonitrile from –10 to+10%. The resolution in the robustness study was not less than <2.0 in all conditions. The stability of the standard solutions and the sample solutions was tested at intervals of 48hours and 10th day. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 10th day for Valganciclovir hydrochloride was 0.53%. The assay values were within 1.5% after 10th day. The results

indicate that the solutions were stable for 10th day at ambient temperature.

**Ruggedness**

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the precision study are tabulated as below table-6. The % RSD values are less than 2.

**RESULTS AND DISCUSSION**

Optimization of chromatographic conditions the primary target in developing this stability indicating HPLC method is to achieve the resolution between Valganciclovir hydrochloride and its degradation products. To achieve the separation of degradation products we used stationary phase C-8 and combination of mobile phase consist of sodium dihydrogen phosphate buffer: Acetonitrile in the ratio (60:40 v/v), The separation of the degradation product and Valganciclovir hydrochloride was achieved on Hypersil C8 stationary phase and the combination of mobile phase consist of sodium dihydrogen phosphate buffer: Acetonitrile in the ratio (60:40 v/v), The tailing factor obtained was less than two and retention time was about 3.675 min for the main peak and less than 4 min for the degradation products, which would reduce the total run time and ultimately increase the productivity thus reducing the cost of analysis per sample. Forced degradation study showed the method is highly specific and entire degradation products were well resolved from the main peak. The developed method was found to be specific and method was validated as per ICH guidelines.

**Table 2: Summary of forced degradation results of Valganciclovir hydrochloride.**

| Stress conditions | Time/h | Peak area | Assay of active % | Degradation/ % substance |
|---|---|---|---|---|
| Acid hydrolysis | 48hours | 912127 | 95.09% | 4.91% |
| Base hydrolysis | 48hours | 925182 | 96.45% | 3.55% |
| Heat | 75˚C | 911927 | 95.07% | 4.93% |
| UV | $10^{th}$ day | 912821 | 95.16% | 4.84% |

**Table 3: Results of recovery of Valganciclovir hydrochloride.**

| Level of addition/ % | Amount added (µg/ml) | Amount found | Avg.Recovery/ % | %RSD |
|---|---|---|---|---|
| 50 | 4 | 11.91 | 99.86 | 1.271 |
| | | 12.16 | | |
| | | 11.88 | | |
| 100 | 8 | 16.28 | 101.42 | 0.32 |
| | | 12.17 | | |
| | | 16.23 | | |
| 150 | 12 | 19.71 | 99.43 | 1.049 |
| | | 19.82 | | |
| | | 20.12 | | |

**Table 4: Results of Precision of Valganciclovir hydrochloride.**

| | INTRA-DAY STUDIES | | | INTER-DAY STUDIES | | |
|---|---|---|---|---|---|---|
| SI.NO | NAME | RT | AREA | NAME | RT | AREA |
| 1 | Injection-1 | 3.674 | 959197 | Injection-1 | 3.674 | 959197 |
| 2 | Injection-2 | 3.678 | 961923 | Injection-2 | 3.672 | 952345 |
| 3 | Injection-3 | 3.654 | 943126 | Injection-3 | 3.684 | 978453 |
| 4 | Injection-4 | 3.682 | 974587 | Injection-4 | 3.668 | 945824 |
| 5 | Injection-5 | 3.686 | 984232 | Injection-5 | 3.686 | 954562 |
| 6 | Injection-6 | 3.676 | 964568 | Injection-6 | 3.676 | 963898 |
| AVG | | 3.675 | 964605.5 | AVG | 3.676667 | 959046.5 |
| STDEV | | 0.011153 | 14018.9 | STDEV | 0.007005 | 11313.95 |
| %RSD | | 0.302 | 1.45 | %RSD | 0.19 | 1.179 |

**Table 5: Linearity of Valganciclovir hydrochloride.**

| Linearity Level (%) | Concentration (µg/ml) | Retention time (min) | Peak area (mv) |
|---|---|---|---|
| 25 | 2 | 3.674 | 239799 |
| 50 | 4 | 3.678 | 489598 |
| 75 | 6 | 3.654 | 719397 |
| 100 | 8 | 3.674 | 959197 |
| 125 | 10 | 3.676 | 1178996 |
| 150 | 12 | 3.672 | 1438795 |

*indicates average of six determinations,

**Table 6: Results of Ruggedness of Valganciclovir hydrochloride.**

| Analysts | Mean area* ± Standard deviation | %RSD |
|---|---|---|
| Analyst 1 | 964605 ± 14018 | 1.45 |
| Analyst 2 | 959546 ± 10660 | 1.179 |

*indicates average of six determinations,

**Table 7: System suitability parameters.**

| Parameters | Valganciclovir hydrochloride |
|---|---|
| Linearity | 2-12 µg/ml |
| Regression equation | y = 118614x + 7332.9 |
| Correlation coefficient | $R^2$ = 0.9996 |
| Retention time | 3.675 |
| Limit of detection(LOD) | 0.076 |
| Limit of quantification(LOQ) | 0.232 |

**Table 8: Analysis of marketed tablets**

| Formulation | Content | Amount found | % Recovery |
|---|---|---|---|
| Valcyte | 450mg | 450.85 | 100.18 |

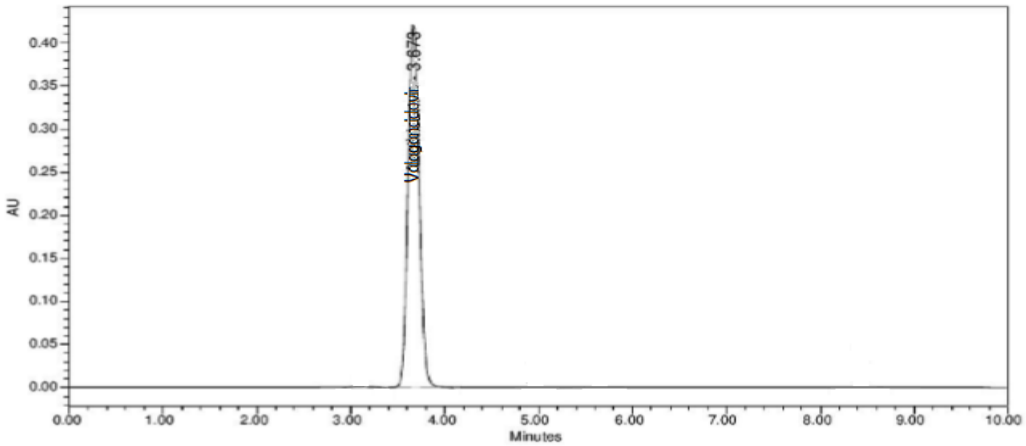


**Figure 2: Sample Chromatogram of standard solution of Valganciclovir hydrochloride.**

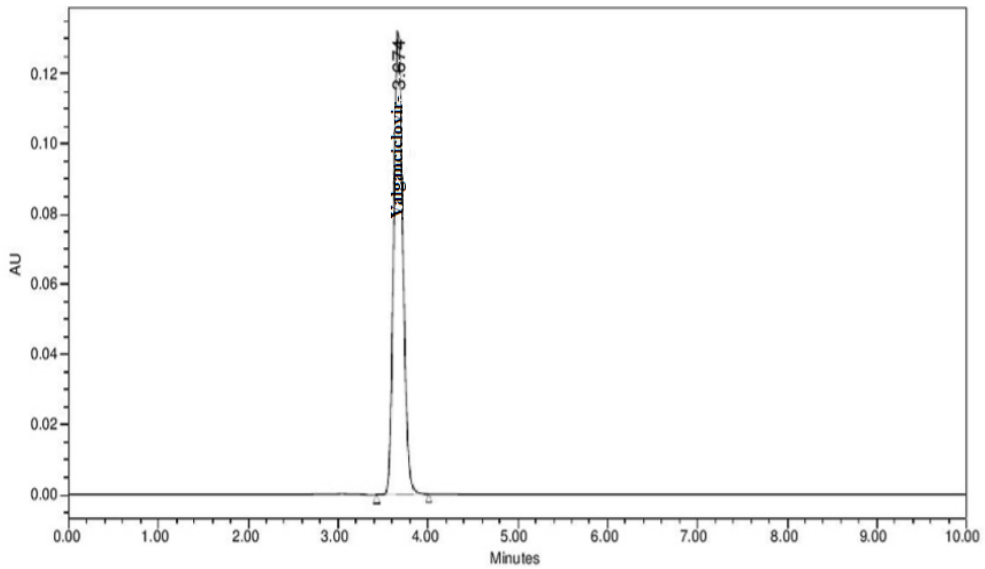


**Figure 3: Chromatogram of Valganciclovir in Formulations.**

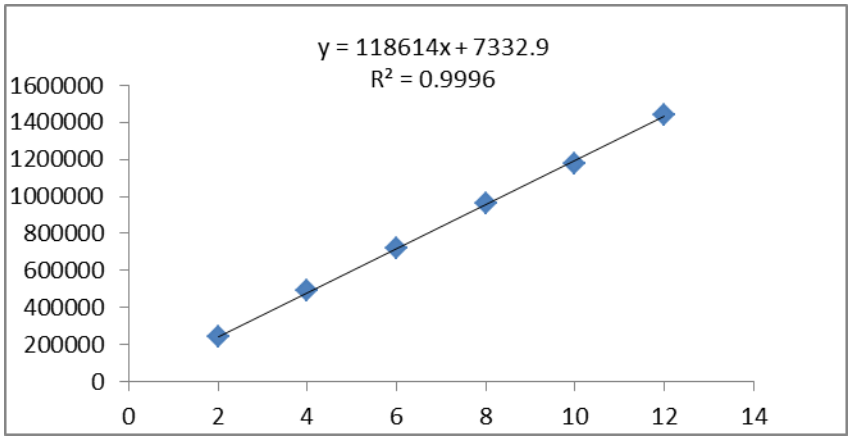


**Figure 4: linearity of Valganciclovir hydrochloride**

**Result of forced degradation experiments**

Degradation was observed for Valganciclovir hydrochloride samples during stress conditions like heat, UV light, base and acid. Valganciclovir hydrochloride was degraded into acid, base, Heat and UV forms polar impurities. In the acidic condition 4.91%, in the basic condition 3.55% after 48 h, in the heat condition 4.93% and in the UV condition 4.84% after 10$^{th}$ day. Degradation was observed for Valganciclovir hydrochloride. Peak purity results greater than 990 indicate that the Valganciclovir hydrochloride peak is homogeneous in all stress conditions tested. The unaffected assay of Valganciclovir hydrochloride in the tablets confirms the stability indicating power of the method the summary of forced degradation results were tabled in Table -2.

## CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of Valganciclovir hydrochloride bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, and rugged and precise. This method can be conveniently used for assessing stability assay of selected substances and dissolution of tablets containing Valganciclovir hydrochloride in quality control laboratory. The study showed that the drug is moderately degraded in acid (4.91%), base (3.55%) heat (4.93%) conditions but highly degraded in the UV (4.84%).

## CONFLICT OF INTERESTS

Declared None.

## ABBREVATIONS

**HPLC:** High performance liquid chromatography;
**ICH:** International council for Harmonization;
**LOD:** Limit of detection;
**LOQ:** Limit of quantitation;
**RSD:** Relative standard deviation.

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