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# Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Valganciclovir Hydrochloride (RS).

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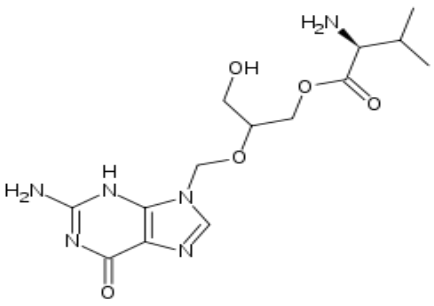
## ABSTRACT

A specific, accurate, precise and sensitive stability indicating RP HPLC method was developed and validated for the determination of Valganciclovir hydrochloride (RS) in bulk drug and pharmaceutical dosage form in the presence of its degradation products. An isocratic RP HPLC method was developed with a Hibar $C_{18}$ (250 X 4.6 mm i.d., 5µ) and methanol: 25mM Ammonium acetate (pH 3.0, adjusted with acetic acid) in the ratio of 10:90 % v/v as mobile phase. The flow rate was maintained at 1 mL $min^{-1}$ and detection was carried out using PDA detector (254 nm). The drug was subjected to stress conditions of degradation in aqueous solutions including hydrolysis, oxidation and photolysis. Degradation was carried out for 24 hrs at 60°C. The drug was found to degrade extensively under alkaline hydrolysis and oxidation with hydrogen peroxide. Mild degradation was observed in neutral but the drug was stable to photolysis. The developed method was validated with respect to linearity, precision, accuracy, ruggedness and specificity.

## INTRODUCTION

Valganciclovir hydrochloride is chemically 2-[(2-amino-6-oxo-6, 9-dihydro-3*H*-purin-9-yl) methoxy] -3-hydroxypropyl (2*S*)-2-amino-3-methylbutanoate. It is an L-valyl ester (prodrug) (*Fig 1)* of ganciclovir that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases, which have demonstrated anti-viral activity against cytomegalovirus infections. Valganciclovir is available as tablet dosage form in the market. The drug was official in USP NF[1]. Previous study on the reactivity of valganciclovir in aqueous solutions proved that the drug is susceptible to degradation in aqueous solutions with different pH [2]. Literature survey reveals that several analytical methods viz UV spectrophotometry [3] and isocratic HPLC[4] were reported for the determination of valganciclovir in bulk and pharmaceutical dosage form. Three LC/MS/MS methods were also reported for the determination of Valganciclovir and its active metabolite ganciclovir in human and rat plasma [5,6,7].




. HCl

**Figure 1: Chemical structure of Valganciclovir Hydrochloride**

The stability-indicating assay methods (SIAMS) are employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the

requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation products. The ICH guideline Q1A (R2) emphasizes that the testing of those features which are susceptible to change during storage and is likely to influence quality, safety and efficacy must be done by validated stability indicating testing method. As per Q1 (R2) information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation [8]. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

We have developed a rapid, simple, robust and economic isocratic RP HPLC method for separating the peaks due to the degradation products from the main peaks of Valganciclovir (RS). The method may be useful for routine analysis in quality control because of low cost and specificity. This method may also be useful for assessing the stability of Valganciclovir (RS) in pharmaceutical preparations.

## EXPERIMENTAL METHOD

### Chemicals, Reagents and solutions

Reference standard of Valganciclovir Hydrochloride was received as gift sample from Dr. Reddy's Laboratories, Hyderabad, India. HPLC grade Methanol, acetic acid, Ammonium acetate and all other chemicals and solvents were procured from Qualigen Fine chemicals (Mumbai, India). High quality pure water was prepared by a Millipore purification system.

### Chromatography

Chromatographic separation and quantitative determination were performed using a gradient ultra fast liquid chromatography (UFLC) containing a Shimadzu gradient pump (LC-20 AD solvent delivery system), a PDA detector (SPD-M20A, Shimadzu), a rheodyne 7120 with a 20 µl loop. As the stationary phase, Hibar Purospher STAR RP-18 column, 5µ particle size, 250 X 4.6 mm (Merck, Darmstadt, Germany) was used. The mobile phase consisted of 10 volumes of methanol and 90 volumes of ammonium acetate, 25 mmol $L^{-1}$ (adjusted to pH 3.0 using acetic acid). The flow rate of the mobile phase was 1.0 mL $min^{-1}$. The wavelength of the PDA detector was set at 254 nm.

### Method validation

The RP HPLC method was validated according to ICH guidelines [9].

*Selectivity*

Selectivity was examined for the non degraded and degraded samples: The solution of Valganciclovir hydrochloride(RS) were stressed under conditions of hydrolysis (acid, base, neutral) at 60°C, Photolysis (solution and solid, exposed to sunlight for 24 hrs) and oxidation (3 & 30 % $H_2O_2$). The samples designed for degradation studies under each condition were placed into three separate flasks and then required volumes of 1N HCl, 0.01N NaOH, 3 & 30% $H_2O_2$ respectively were added to them. Each determination was carried out three times.

*Linearity*

The linearity plot of the method was obtained in the concentration range of 18 – 72 µg $mL^{-1}$.

### Accuracy

The accuracy of the method was determined by recovering Valganciclovir from the placebo. The recovery test was performed at three levels, namely, 60, 100 and 140% of the nominal concentration of Valganciclovir hydrochloride during degradation studies. Three samples were prepared for each recovery levels. The solutions were analyzed and the percentage recovery was calculated from the calibration curve.

*Precision*

The precision of the assay was determined in relation to repeatability (intraday) and intermediate precision (inter day). For the evaluation of repeatability of the methods, six samples at 100% concentration level was

determined during the same day for Valganciclovir hydrochloride. Intermediate precision was studied by comparing the assays performed on three different days.

*Limit of detection (LOD) and Limit of quantification (LOQ)*

The LOD and LOQ parameters were determined based on signal to noise ratio. The detection limit is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3) and quantification limit is the smallest concentration of the analyte that can be reliably quantified with acceptable accuracy and precision.

*Robustness*

The robustness of the procedure was evaluated after changing the following parameters: the composition of mobile phase (content of methanol in the range of 0.1 – 1.0%); the mobile phase flow rate (flow rate in the range of 0.9 – 1.1 mL $min^{-1}$); pH of the buffer (pH in the range of 2.8 – 3.2) and concentration of the buffer (buffer concentration in the range of 20 -30 mmol $L^{-1}$). For each parameter change, its influence on the retention time, resolution and peak shape were evaluated.

## Forced degradation study of Valganciclovir hydrochloride

*Degradation in aqueous solutions*

The degradation of Valganciclovir hydrochloride in aqueous solution was studied in hydrochloric acid (0.1mol $L^{-1}$ at room temperature & 1 mol $L^{-1}$ for 24 hrs at 60˚C), in sodium hydroxide (0.1mol $L^{-1}$ at room temperature & 0.01 mol $L^{-1}$ for 24 hrs at 60˚C) and water (room temperature & for 24 hrs at 60˚C). Degradation was initiated by dissolving 2.5 mg of the drug in 25.0 mL of the solutions in stoppered flasks. At specified time, samples of the reaction solutions (1.0 mL) were instantly cooled with a mixture of ice and water and neutralized. The samples were then analyzed by the developed RP HPLC method.

*Oxidative degradation*

2.5 mg of Valganciclovir hydrochloride was accurately weighed in 25.0 mL, dissolved in diluents (water) and the volume was made with 3% and 30% v/v $H_2O_2$. The solution was then subjected to oxidative degradation at room temperature and for 24 hrs at 60˚C (3% & 30%). At specified time, samples of the reaction solutions (1.0 mL) were instantly cooled with a mixture of ice and water. The samples were then analyzed by the developed RP HPLC method.

*Photolysis*

Ten milligram of the sample was weighed into a petridish. In order to achieve degradation, the sample in solid state and solution state were exposed to sunlight for 24 hrs. The samples were then suitably dissolved in methanol and subjected for analysis.

# RESULTS AND DISCUSSION

## Optimization of chromatographic conditions

Optimization of the method was carried out using various concentration of methanol while keeping the pH and molarity of the aqueous phase constant. A solvent combination of 25 mmol $L^{-1}$ Ammonium acetate (pH 3.0, adjusted with acetic acid): methanol (90:10 v/v) on a Hibar Purospher STAR RP-18 column (5µ particle size, 250 X 4.6 mm) as stationary phase gave satisfactory separation of R and S diastereomers of Valganciclovir hydrochloride and their degradation products formed under various stress conditions. The detection was carried out at 254 nm using PDA detector. The mobile phase flow rate was 1.0 mL $min^{-1}$. Typical retention times of Valganciclovir hydrochloride R and S were about 10.1 min and 11.4 min, respectively (*Fig 2*). In blank sample purities of R and S peaks were 99.86 and 99.34%.

## Method validation

The method was validated for parameters such as specificity, linearity, precision, accuracy and robustness.

Photodiode array detection was used as evidence of the specificity of the method and to evaluate the homogeneity of Valganciclovir hydrochloride R and S. The peak purity values were more than 99.00% for R and S diastereomers at 254 nm, which proves that the peaks of the degradants were not interfering with the main peak.

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Linearity of the method was tested for 40% to 160% of the targeted level of the assay concentration (18, 27, 36, 45, 54, 63 and 72 µg $mL^{-1}$). Linearity solutions were injected in triplicate. The calibration plots were linear in the concentration range of 18 – 72 µg $mL^{-1}$. The calibration curves were described by the equation Y=mX+c; i.e. Y = 38440X + 250538. The calibration curves were constructed on six different days over a period of two weeks to determine the variabilities of slope and intercept. The interday variability of slope and intercept were not significant. The intraday and interday precision values were calculated for one concentration at 100% level (Table 1). The RSD values <1% demonstrate that the method was precise.

**Table 1: Intraday and Interday precision studies**

| Intraday studies<br>Peak areas of Valganciclovir Hydrochloride (45 µg $mL^{-1}$) | | Interday studies<br>Peak areas of Valganciclovir Hydrochloride (45 µg $mL^{-1}$) |
|---|---|---|
| 1950990<br>1966074<br>1964011<br>1983435<br>1971961<br>1975544<br>Mean : 1968669<br>RSD : 0.564 | Day 1 | 1956589<br>1965287<br>1945282<br>1968551<br>1958625<br>1965522<br>Mean : 1959976<br>RSD : 0.434 |
| | Day 2 | 1968545<br>1964252<br>1954785<br>1956664<br>1965454<br>1958884<br>Mean : 1961431<br>RSD : 0.278 |
| | Day 3 | 1966543<br>1962323<br>1954522<br>1961785<br>1954875<br>1968542<br>Mean : 1961432<br>RSD : 0.296 |

Recovery was performed at three levels, 60, 100 and 140% of the label claim for six times. Good recoveries were obtained for each concentration, confirming that the method was accurate (Table 2).

**Table 2: Accuracy and Recovery studies of Valganciclovir Hydrochloride (n=3)**

| Drug | Amount Added (µg/mL) | Amount found (µg/mL) | % Recovery ± RSD |
|---|---|---|---|
| Valganciclovir Hydrochloride | 27 | 26.87 ± 0.38 | 99.51 ± 0.75 |
| | 45 | 44.62 ± 0.25 | 99.16 ± 0.51 |
| | 63 | 62.75 ± 0.49 | 99.60 ± 0.43 |

Under the applied chromatographic conditions the LOD and LOQ of Valganciclovir hydrochloride was 50 and 150 ng $mL^{-1}$ respectively (Table 3).

**Table 3: System suitability parameters**

| Parameters | Valganciclovir HCl |
|---|---|
| Linearity and Range | 18-72 µg $mL^{-1}$ |
| Regression equation | Y= 38440x + 250538 |
| Correlation coefficient | 0.9988 |
| Theoretical plates | 145897625 |
| Resolution factor | 2.02 |
| Asymmetric factor | 1.02, 0.99 |
| Limit of Detection(LOD) | 50 ng $mL^{-1}$ |
| Limit of Quantification(LOQ) | 150 ng $mL^{-1}$ |

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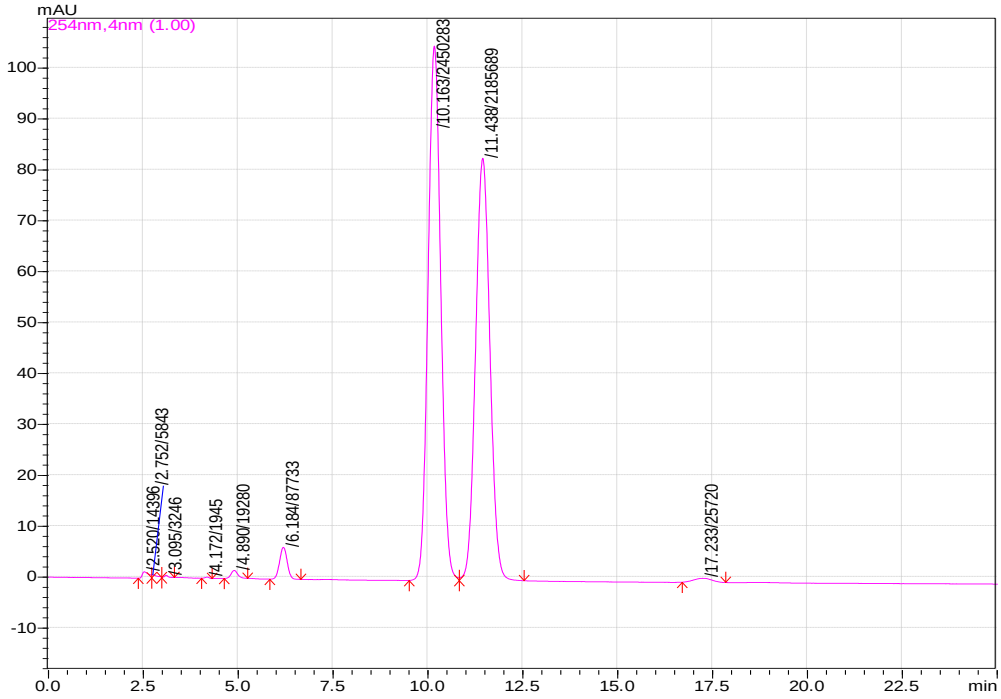


**Figure 2: Typical Chromatogram of Standard drug of Valganciclovir Hydrochloride**

Robustness of the procedure was evaluated by changing the following parameters: Composition of mobile phase, flow rate, pH and buffer concentration. With change of each parameter, the effects on the retention time, resolution and peak shape were evaluated. No significant changes in the resolution and peak shapes were detected.

### Result of forced degradation experiments

According to the draft guideline of 1998 stability indicating methods are defined as "validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products and other components of interest can be accurately measured without interference".[8] S Singh and Bakshi suggested that, during stability studies, degradation of 20-80% should be achieved for establishing the stability indicating nature of the assay.[10] The drug Valganciclovir hydrochloride was found to undergo extreme degradation in basic hydrolysis and oxidation. It was observed that 95 – 100% of Valganciclovir hydrochloride was found to degrade in basic hydrolysis and oxidation with 30% $H_2O_2$. In acidic conditions, it was observed that the drug was found to show 4% degradation at room temperature with 0.1 M Hydrochloric acid. As suggested by Singh and Bakshi [10], to achieve 20% degradation the drug was refluxed with 1 M Hydrochloric acid for 24 hrs at 60˚C. About 25% of degradation was observed with two additional peaks at the retention time of 2.7 and 6.0 min respectively. Valganciclovir hydrochloride was found to be highly susceptible to basic hydrolysis. Almost complete degradation (99.6%) was observed when the drug was incubated with 0.1 M sodium hydroxide at room temperature for 2 hours. To minimize the effect of basic hydrolysis, the drug was refluxed with 0.01M sodium hydroxide for 24 hrs at 60˚C. Almost 50% degradation was observed after 2 hrs and complete degradation was observed after 12 hrs. The drug was more stable to neutral hydrolysis. When an aqueous solution was subjected to stress conditions i.e. reflux for 24 hrs at 60˚C, only 14% degradation was observed. In oxidative degradation, it was observed that the drug was found to show 5% degradation at room temperature with 3% hydrogen peroxide and 10% degradation when refluxed at 60˚ C for 24 hrs. Further, the concentration of hydrogen peroxide was increased to 30% and refluxed for 24 hrs. Thus, 100 % degradation was observed with two additional peaks at 2.7 and 6.0 min. Valganciclovir hydrochloride was not susceptible to photolysis as the degradation was less than 10% in solution as well as in solid state. Chromatograms of the solutions obtained after degradation under acidic, basic, neutral, oxidation and photolysis are shown in *Fig 3-7*. The results of forced degradations under various conditions are summarized in Table 4 & 5. Additional peaks at the retention time of 2.7 and 6.0 min were found in all stressed conditions suggesting the formation of two degradants.

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Table 4: Results of degradation studies at room temperature

| Stress conditions | % Degradation at room temperature after 2 hrs |
|---|---|
| 0.1M HCl | 4.01 |
| 0.1M NaOH | 99.64 |
| Neutral hydrolysis | 0.48 |
| 3% $H_2O_2$ | 5.95 |
| Photolysis (Solid state) | 1.83 (After 24 hrs) |
| Photolysis (solution state) | 9.03 (After 24 hrs) |

Table 5: Results of degradation studies for 24 hrs at 60˚C

| Time | % Degradation (Stress conditions) | | | | |
|---|---|---|---|---|---|
| | 1M HCl | 0.01M NaOH | Neutral | 3% $H_2O_2$ | 30% $H_2O_2$ |
| 2 | 1.68 | 46.22 | 6.82 | 0.91 | 65.98 |
| 4 | 16.49 | 51.03 | 7.93 | 5.51 | 84.99 |
| 6 | 18.88 | 52.64 | 12.38 | 6.31 | 93.12 |
| 8 | 21.39 | 53.63 | 12.72 | 6.24 | 96.88 |
| 12 | 25.04 | 100 | 13.27 | 6.85 | 100 |
| 24 | 25.76 | 100 | 14.15 | 10.52 | 100 |

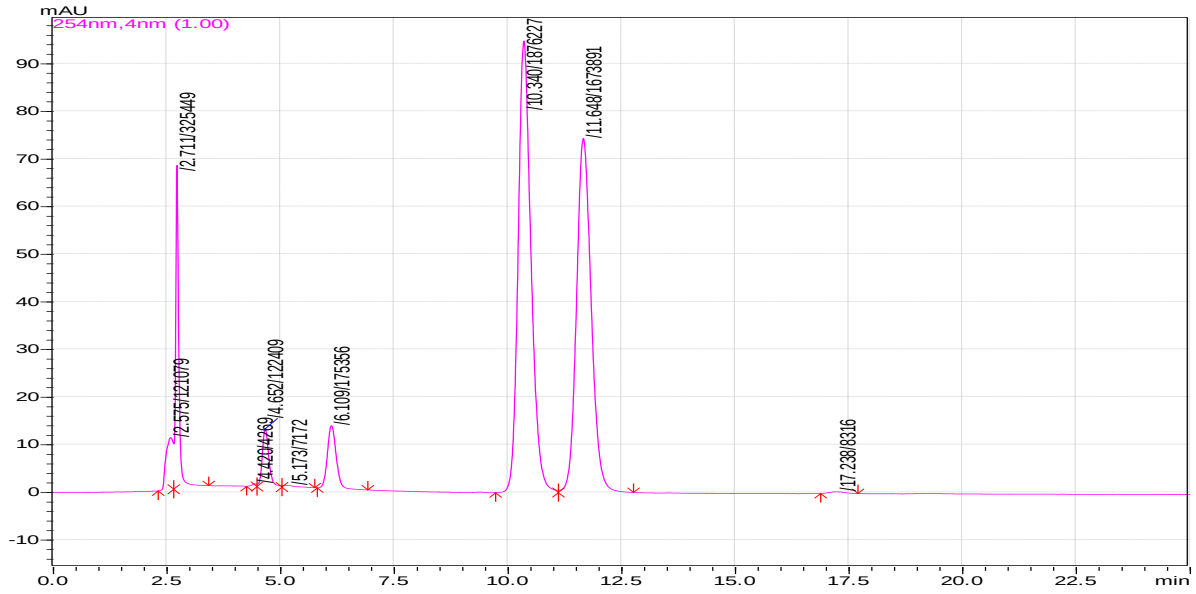


Figure 3: Typical chromatogram of Acid degradation sample with 1M HCl under reflux at 60°C for 24 hours

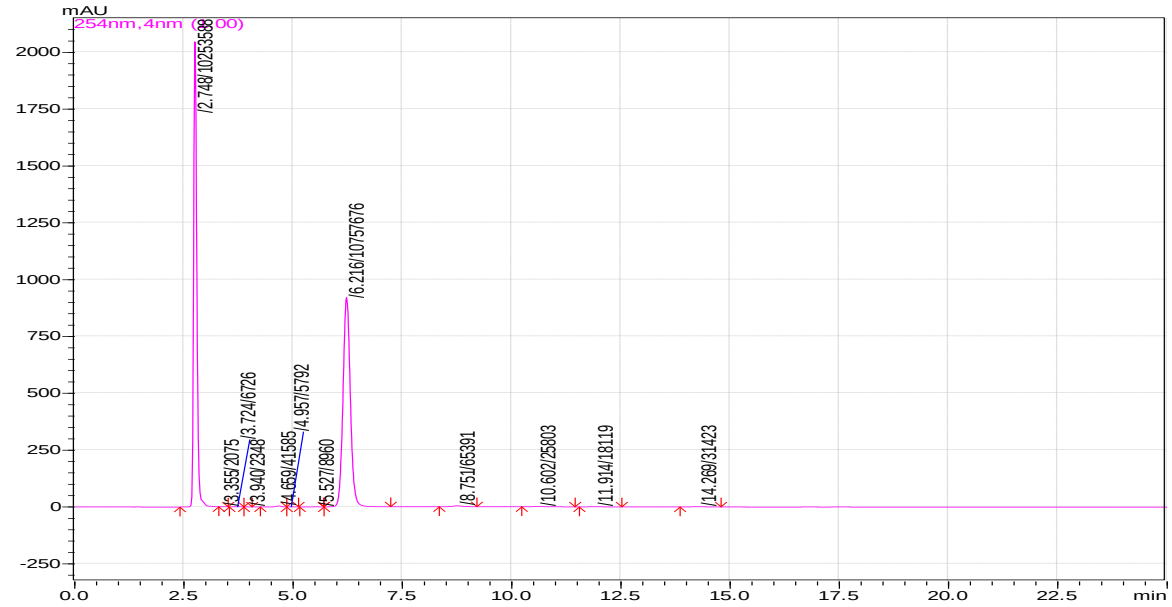


Figure 4: Typical chromatogram of Base degradation sample with 0.01M NaOH under reflux at 60°C for 24 hours

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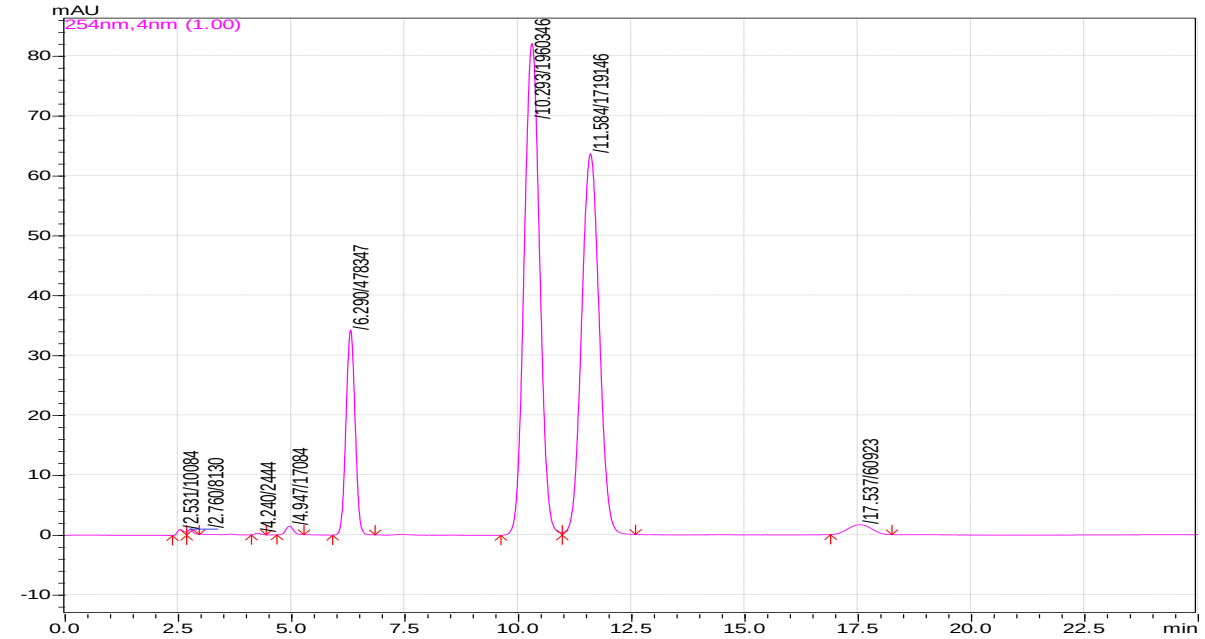


Figure 5: Typical chromatogram of Neutral degradation sample with water under reflux at 60°C for 24 hours

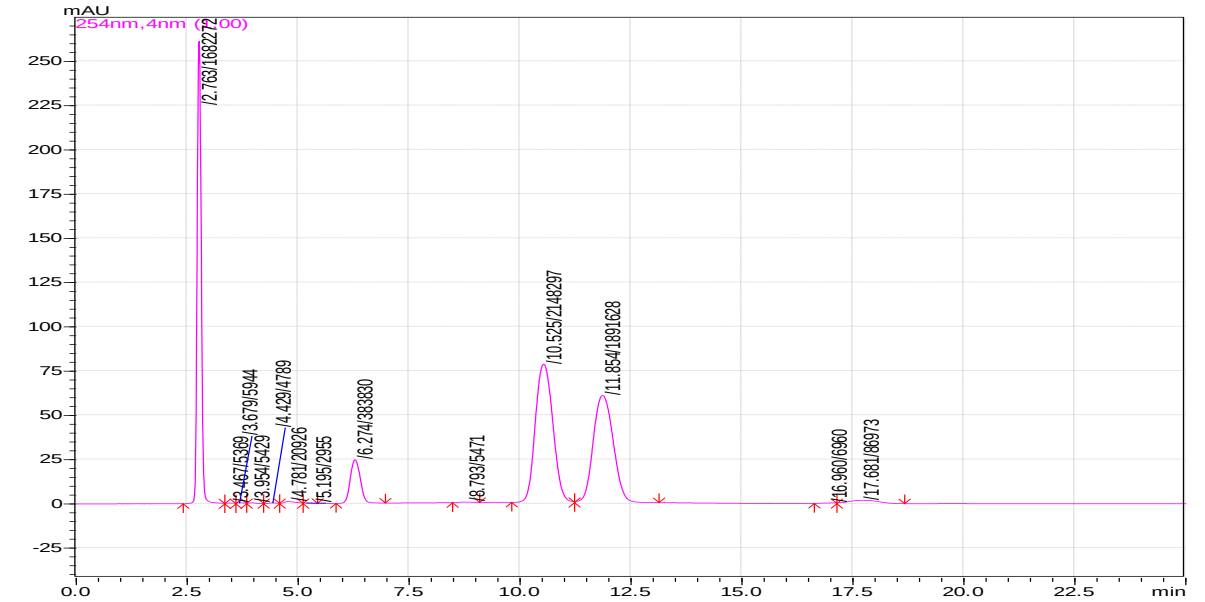


Figure 6: Typical chromatogram of oxidative degradation sample with 3% hydrogen peroxide under reflux at 60°C for 24 hours

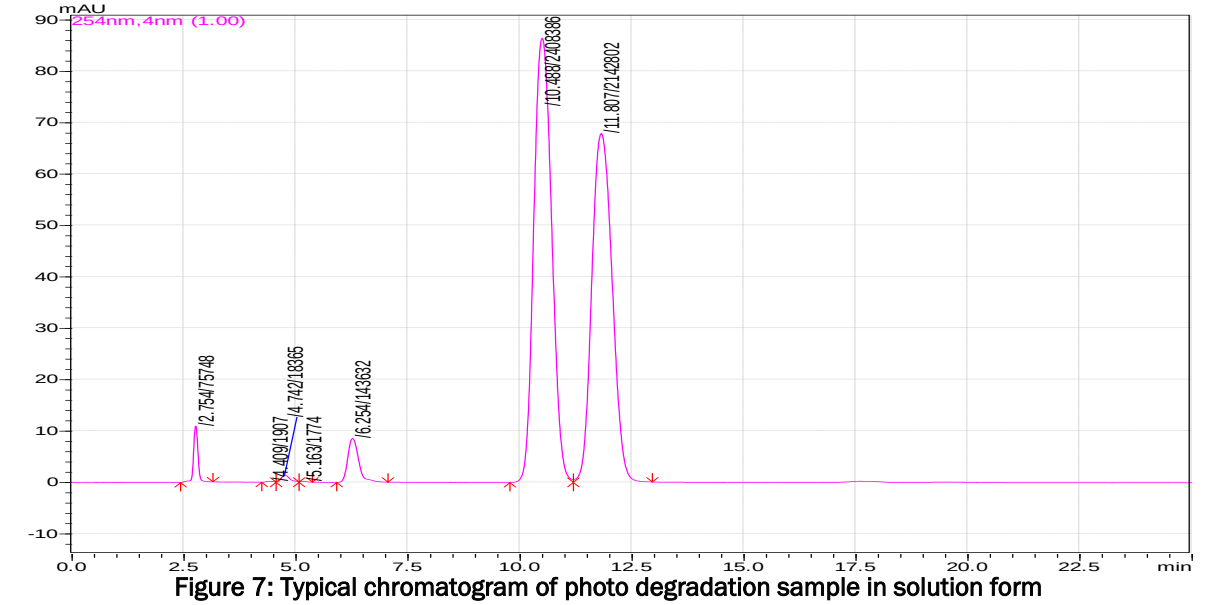


Figure 7: Typical chromatogram of photo degradation sample in solution form

Valganciclovir hydrochloride chemically is an aminoacyl ester. Protonation of the amino group may greatly enhance the rate of hydrolysis of Valganciclovir hydrochloride to ganciclovir under physiological conditions. The degradation of the drug may correspond to ganciclovir and valine. The peak at the retention time of 2.7 min was confirmed to be valine as standard valine eluted at the same retention time under the optimised experimental conditions.

Additionally the developed HPLC method was also applied for the estimation of Valganciclovir hydrochloride in pharmaceutical formulations.

## CONCLUSION

For the estimation of Valganciclovir hydrochloride in the presence of their degradation products, an RP HPLC method was developed. For the selected parent and related substances formed different elution orders were observed, which demonstrated different structures of the degradation products. The drug was found to be more susceptible to hydrolysis under basic conditions and oxidation. The developed method was also validated for the various validation parameters. The method also provides better resolution between R and S diastereomers of Valganciclovir hydrochloride. It may be concluded that the developed method was selective for the determination of Valganciclovir hydrochloride in the presence of various degradation products. Moreover the developed RP HPLC method was found to be more precise, reliable, sensitive, and accurate. The method can be used for routine analysis in quality control because of the short run time and low cost.

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