Research Article

Accelerated Stability Studies on Valacyclovir Hydrochloride by RP-HPLC

VMK. Gowtham Potnuru, C. Jothimanivannan*, CH. Arjun, M. Jambulingam, S. Ananda Thangadurai and D. Kamalakannan

Swamy Vivekanandha College of Pharmacy, Department of Pharmaceutical Analysis. Elayampalaym, Tiruchengode, Namakkal, Tamilnadu, India.

ABSTRACT

A specific, accurate, precise and sensitive RP-HPLC method has been developed for the determination of Valacyclovir hydrochloride in the presence of its degradation products by accelerated stability studies. As per ICH guideline Q1A (R2), drug was subjected to all stress conditions such as hydrolysis (acidic and alkaline), oxidation (30% H_2O_2 v/v), photolysis, (As per ICH guideline Q1B), thermal degradation and humidity study. All stressed samples were successfully analyzed on C_{18} column using mobile phase water: methanol: perchloric acid in the ratio of 95:4:1v/v with a flow rate of 0.75 ml/min and detection was made at 255 nm. Moderate change in stability of drug was shown in thermal and oxidative studies whereas drug was found to be stable in acid, alkali, humidity and photolysis studies. The developed method was validated over the linearity, precision, accuracy and specificity as per ICH guideline Q2B. The major degradants was identified as acyclovir and D-Valacyclovir hydrochloride through comparison with the standard. The developed method with good separation of all degradation products from drug could be successfully applied for the determination of Valacyclovir hydrochloride in the presence of its degradation products in formulation. It can be used for analysis of samples during stability testing.

Keywords: Valacyclovir hydrochloride, RP-HPLC, Forced Degradation.

INTRODUCTION

Valacyclovir is, L-Valine 2-[(2–amino–1, 6–dihydro–6–oxo–9*H*-purin–9yl) methoxy] ethyl ester (Fig 1). After oral administration valacyclovir is rapidly converted into acyclovir which has demonstrated antiviral activity, against herpes simplex virus type I (HSV-1) and 2 (HSV-II), Varicella zoster virus (VZV). Valacyclovir is available as tablet dosage form in the market. Few HPLC methods were reported for the determination of valacyclovir in its pharmaceutical formulation and in serum. Previously reported HPLC methods using C_{18}, C_8 with flow rate 3, 1 ml/min respectively our reported liquid chromatographic method determined valacyclovir in presence of acyclovir using C_{18} with flow rate 0.75 ml/min[1-8]. The ICH guideline Q1A (R2)[9] emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy, must be done by validated stability indicating testing method. As per Q1A (R2) information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation. 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. Stress testing is likely to be carried out on a single substance[10]. The main objective was to develop a suitable method of analysis which is stability indicating and to get an idea of how drug substance or product degrades, degenerates and behaves under changing conditions by keeping all this in view, it was thorough worthwhile to develop stability indicating HPLC method for Valacyclovir pure drug and its tablet dosage form. Literature survey revealed that few methods are reported for the determination of Valacyclovir in the presence of its degradation product in pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and Reagents:

Valacyclovir hydrochloride (standard) is obtained from Aurobindo Pharma Ltd., Hyderabad, A.P, India. Methanol, Perchloric acid, Water, Hydrogen peroxide, Hydrochloric acid, Sodium hydroxide etc., which are of analytical grade reagent were purchased from E. Merck Ltd., Mumbai, India. Double distilled water was used throughout the study. Pharmaceutical dosage form (tablet) was obtained from local market, manufactured by Cipla Pharma Ltd., as a brand name of Valcivir 500 mg which was used as sample.

Instrumentation

Analysis was performed on a chromatographic system of Waters 2629 equipped with an auto injector with UV/Visible detector (UV-2489). A chromatographic separation was achieved on Crownpak C_{18} column (5µm, 250mm x 4.6mm, i.d) analytical column. Data acquisition was made with Empower-2 software. Analytical Balance (BSA224S-CW, Sartorius), pH Meter (Eutech) from Shimadzu were used for the study. Thermal oven and humidity desiccator were used to find stability of drug.

Chromatographic conditions

Valacyclovir hydrochloride chromatogram was developed on Crownpak C_{18}, (250mm x 5 µm, 4.6 mm, i.d) column, using a mobile phase containing water: methanol: perchloric acid in the ratio of 95:4:1v/v at ambient temperature. The flow rate was maintained at 0.75 ml/ min throughout analysis. Initially the method was developed for standard drug then it was extended to stress samples. The standard and all stress samples were prepared in mobile phase.

Preparation of Standard Stock Solutions

The quantity containing 60 mg of Valacyclovir hydrochloride was accurately weighed and transferred into a 100 ml dry, clean volumetric flask and the volume was made up to mark with diluent (600 µg/ml). 2 ml was pipetted out from the above solution in to a 10 ml dry, clean volumetric flask and the volume was made up to mark with mobile phase (120 µg/ml). The solution was filtered and sonicated to remove water bubbles. Twenty micro liters of solution was injected into system, the chromatogram obtained was shown in Figure 2.

Sample preparation

Ten tablets were accurately weighed and powdered. The equivalent weight of 60 mg of tablet powder was transferred into a 100 ml dry, clean volumetric flask and the volume made up to the mark with diluent (600 µg/ml). From this 2 ml was pipetted out in to a 10 ml dry, clean volumetric flask and the volume was made up to mark with mobile phase (120 µg/ml). The solution was filtered and sonicated to remove water bubbles.

Forced degradation studies (stress testing)

Acid degradation

The reaction was initiated by adding 10 ml of Valacyclovir hydrochloride to 100 ml volumetric flask containing 5 M hydrochloric acid. The volume made to 100 ml with 5 M hydrochloric acid. The degradation was carried out in thermostatically controlled water bath, protected from light. The degradation was carried at 85^0c temperature for 60 minutes. The chromatogram obtained was shown in Figure 4.

Base degradation

The reaction was initiated by adding 10 ml of Valacyclovir hydrochloride to 100 ml volumetric flask containing 1 M sodium hydroxide. The volume made to 100 ml with 1 M sodium hydroxide. The degradation was carried out in thermostatically controlled water bath, protected from light. The degradation was carried at ambient temperature for 60 minutes. The chromatogram obtained was shown in Figure 5.

Peroxide degradation

The reaction was initiated by adding 10 ml of Valacyclovir hydrochloride to 100 ml volumetric flask containing 30 % hydrogen

peroxide. The volume made to 100 ml with 30 % hydrogen peroxide. The degradation was carried out in thermostatically controlled water bath, protected from light. The degradation was carried at temperature 85^0C for 30 minutes. The chromatogram obtained was shown in Figure 6.

Thermolytic degradation
The thermal stability of Valacyclovir hydrochloride was studied by heating it, both as powder and in mobile phase solution at temperature 105°C for 24 hours. The chromatogram obtained was shown in Figure 7.

Photolytic degradation
Valacyclovir hydrochloride (60 mg, accurately weighed) was dissolved in 100 ml mobile phase. The solution was exposed to light (UV lamp) for 120 hrs. The chromatogram obtained was shown in Figure 8.

Humidity degradation
Valacyclovir hydrochloride sample was kept under 25^0C temperature at 90 % RH for 120 hrs time in humidity chamber under observation. The chromatogram obtained was shown in Figure 9

Method validation
Validation of developed analytical method was performed as per ICH guideline Q2B[11], over the linearity, accuracy, precision and specificity.

RESULTS AND DISCUSSION

Analysis of stressed samples
Analysis of all stressed samples was performed using a mobile phase containing water: methanol: perchloric acid in the ratio of 95:4:1v/v. The retention time of degraded sample was found to be same as that of undegraded sample for acid, base, photolysis and humidity conditions. So there is no significant effect of acid, base, photolysis and humidity on Valacyclovir hydrochloride sample. But moderate changes in stability of drug were observed in peroxide and thermal conditions. The results were shown in Table 1.

Validation of the method
Method and system precision
Precision of the method was verified by repeatability (system precision) and intermediate precision (method precision) studies. Repeatability studies were performed by six replicate injections of 120 µg/ml of Valacyclovir hydrochloride on the same day. The studies were replicated on different days to determine method precision. The data is quoted in Table 2

Accuracy
Accuracy of the method was carried out by applying the method to drug sample to which known amount of Valacyclovir hydrochloride standard powder corresponding to 50 %, 100 % and 150 % of label claim had been added (standard addition method), the solutions are analyzed by optimized method. In our study, the percentage recovery of Valacyclovir hydrochloride was found to be 100.7%, 101.2%, and 101.3 % from 50 %, 100 % and 150 % sample solutions respectively. The obtained percentage recovery of both drugs was found to be within the range. This indicates the proposed method was more accurate than the existing methods. The results were displayed in Table 3.

Linearity
From the standard stock solution 0.5, 1, 1.5, 2, and 2.5 ml was transferred to five 10 ml flasks and made up the volume with mobile phase. The concentrations of VAL were found to be 30-150 µg/ml. Twenty micro liter of the each standard solution was injected and chromatograms were recorded. The correlation coefficient (r^2) value of drug was found to be 0.999. The equation of regression line for Valacyclovir hydrochloride was found to be y = 0.115x – 0.081.

Specificity
Good separation of Valacyclovir and its degradation products were from each other. A resolution factor for the drug peak was 5.3 from the nearest resolving peak with no interference from the sample matrix proved that the method was found to be specific to the drug.

Stability

The quantity containing 60 mg of Valacyclovir hydrochloride was accurately weighed and transferred into a 100 ml dry, clean volumetric flask and the volume made up to the mark with diluent (600 µg/ml). From this 2 ml was pipetted out in to a 10 ml dry, clean volumetric flask and the volume was made up to mark with mobile phase (120 µg/ml). Twenty micro/liter of above solution was injected at different time intervals of 0, 2nd, 4th, 6th, 8th, 10th and 12th hour. The results were shown in Table 4.

CONCLUSION

The study brings forward new and interesting aspects on the decomposition behavior of Valacyclovir hydrochloride. A stability indicating reversed phase liquid chromatography was developed for determination of Valacyclovir during stability testing as per ICH recommended stress studies. Forced degradation studies revealed that possible degradation products do not interfere with the determination of Valacyclovir hydrochloride. The developed reversed phase liquid chromatographic method was validated over the linearity, precision, accuracy and specificity, proved to be convenient and effective for the determination of Valacyclovir during stability testing of the bulk as well as pharmaceutical tablet dosage form. A specific, accurate, precise and sensitive validated reversed phase liquid chromatographic method has been developed for the determination of Valacyclovir in the presence of its degradation products in bulk drug as well as tablet dosage form. Moreover the lower organic solvent consumption achieves more resolution between degradation products. The method is cost effective when comparative to the other existing methods. The metabolites also determined along with Valacyclovir hydrochloride in various stability conditions such as acid, base, peroxide, thermal, photolytic and humidity.

Conflict of interest statement

We (the authors of this manuscript) have no financial and personal relationships with other people or organizations that could influence our work.

ACKNOWLEDGEMENT

We would like to thank Aurobindo Pharma Pvt., Ltd. Hyderabad, (Andhra Pradesh) for providing Valacyclovir hydrochloride gift sample.

Table 1: Retention time for all degradation products

Retention time			
condition	Acyclovir	D- valacyclovir	Valacyclovir hydrochloride
Undegraded sample	3.271	14.282	26.323
Acid degradation	3.373	14.444	26.512
Base degradation	3.266	14.061	25.554
Peroxide degradation	3.192	13.688	24.755
Thermal degradation	3.304	14.694	27.324
Photolytic degradation	3.299	14.390	26.400
Humidity degradation	3.305	14.445	26.534

Table 2: System Precision and method precision data results for the VAL

	VAL (System Precision)		VAL (Method Precision)	
S.No	AUC	PERCENTAGE	AUC	PERCENTAGE
1	14018628	100	14011457	99.94885
2	14086699	100.48558	14015478	99.97753
3	14017310	99.990598	14065478	100.3342
4	14025310	100.04767	14023568	100.0352
5	14039372	100.14797	14056478	100.27
6	14087189	100.48907	14078456	100.4268
Mean	14045751	100.193	14041819	100.16
S.D	32854	0.2139	26031.07	0.1857
%R.S.D	0.2	0.213488	0.185382	0.185403

Table 3: Accuracy data results for the VAL at the level of 50%,100% and 150%

Assay No.	Concentration (% of label claim)	Amount added (mg)	Amount recovered (mg)	%Recovery (98.0% to 102.0%)	%RSD NMT 2%
1.	50%	1250.0	1258.9	100.7	0.1%
2.	100%	2500.1	2529.6	101.2	0.1%
3.	150%	3750.7	3793.9	101.2	0.1 %

Table 4: Stability data of Valacyclovir hydrochloride

Time interval	Peak area
0th hour	13435257
2th hour	13429019
4th hour	13427836
6th hour	13442353
8th hour	13477004
10th hour	13416052
12th hour	13414453
Mean	13434568
S.D	19586.66
%R.S.D	0.145793

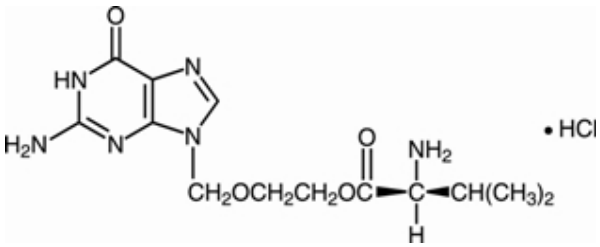

Fig. 1: Valacyclovir hydrochloride

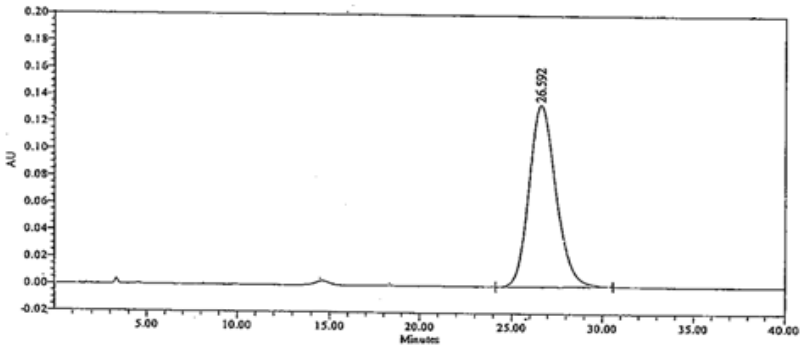

Fig. 2: Chromatogram of Valacyclovir hydrochloride

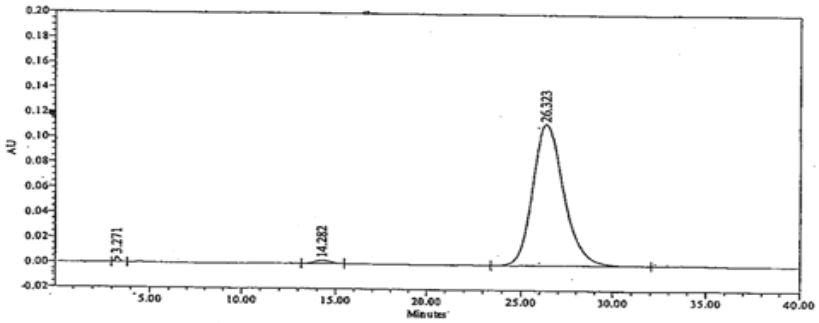

Fig. 3: Chromatogram of undegraded sample

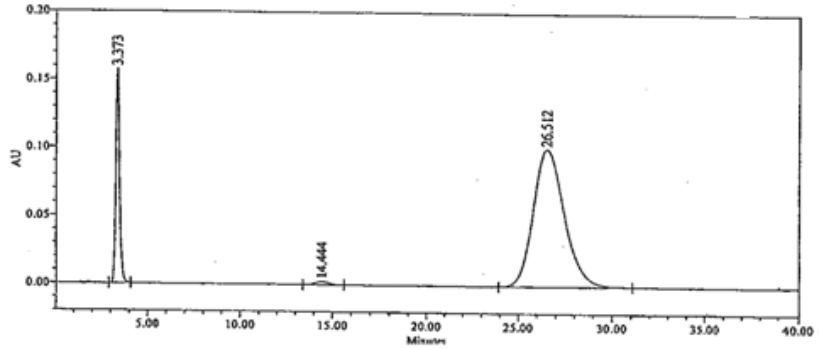

Fig. 4: Chromatogram of acid degradation

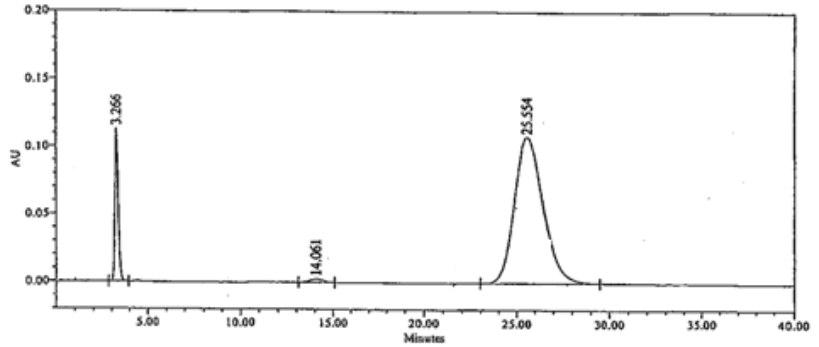

Fig. 5:Chromatogram of base degradation

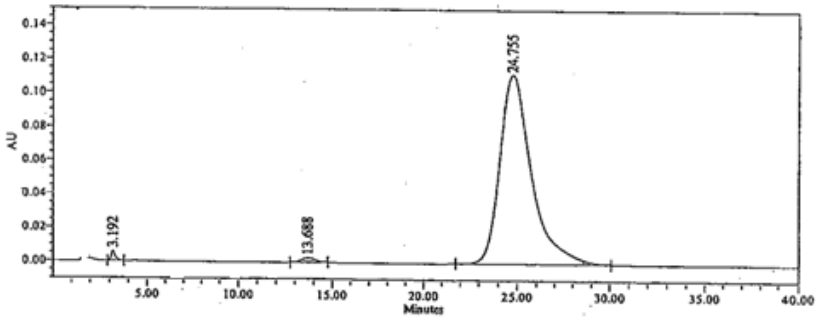

Fig. 6: Chromatogram of peroxide degradation

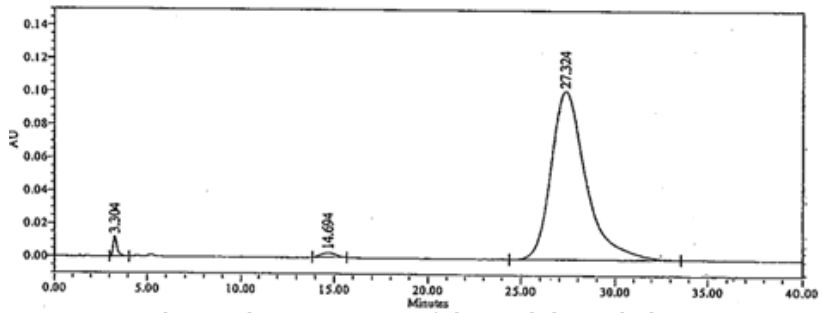

Fig. 7: Chromatogram of thermal degradation

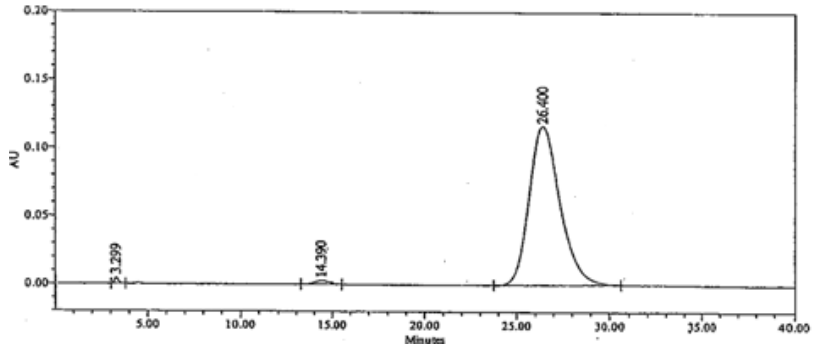

Fig. 8: Chromatogram of photolytic degradation

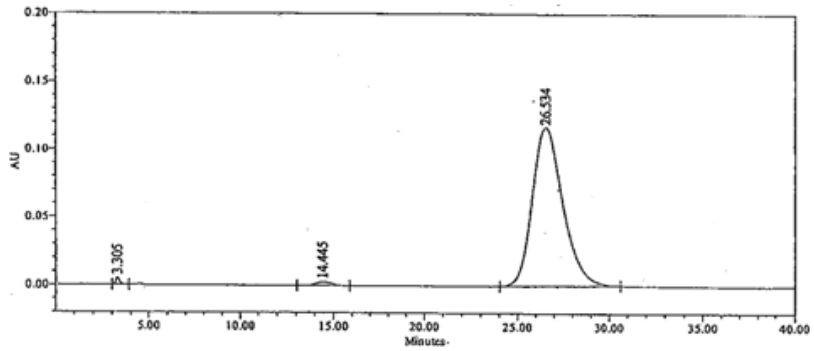

Fig. 9: Chromatogram of humidity degradation

REFERENCES

1. Patil1 GD, Yeole1 PG, Manisha P and Wadher SJ. A validated specific reverse phase liquid chromatographic method for the determination of Valacyclovir in the presence of its degradation products in bulk drug and in tablet dosage form. Int J Chemtech Res. 2009;1(1):16-26.
2. Sudhakar RJ, Maqsood AS, Chakravarthi and Prabhavathi K. Spectrophotometric assay of Valacyclovir in pharmaceutical dosage forms. International Journal of Research and Reviews in Applied Sciences. 2011;8(3):346-348.
3. Sugumaran M, Bharathi V, Hemachander R and Lakshmi M. RP-HPLC method for the

determination of valacyclovir in bulk and Pharmaceutical formulation. Der Pharma Chemica. 2010;3(4):190-194.
4. Ganesh M, Narasimharao CV, Saravana kumar A, Kamalakannan K, Vinoba M, Mahajan HS and Sivakumar T. UV spectrophotometric method for the estimation of valacyclovir hcl in tablet dosage form. E Journal of Chem. 2009;6(3):814-818.
5. Sugumaran M and Jothieswari D. Development and validation of spectroscopic method for estimation of valacyclovir in tablet dosage form. Oriental Journal of Chemistry. 2010;26(1):163-165.
6. Venkata reddy P, and Sudha rani B. Estimation of some antiviral drugs using TPooo as analytical reagent. E Journal of Chem. 2006;3(3):154-158.
7. Siva ramakrishna V, Sudha lakshmi PB, Ravi kumar D and Ram babu C. Spectrophotometric determination of Valacyclovir Hcl through oxidative coupling reaction in bulk and its pharmaceutical preparations. International. Journal of Chemtech Research. 2012;4(1):138-142.
8. ICH Q A1 (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization, IFPMA, Geneva, 2003.
9. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization, IFPMA, Geneva, 2003.
10. ICH, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, IFPMA, Geneva. 1996.