International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.1, No.3 , pp 702-708, July-Sept 2009

Stability-indicating liquid chromatographic method for valacyclovir

K.Srinivasa Rao*and M. Sunil

Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Orissa (India).

E-mail: ksrao108@gmail.com,Ph: 099384950

ABSTRACT: A novel stability-indicating high-performance liquid chromatographic assay method was developed and validated for valacyclovir in the presence of degradation products generated from forced decomposition studies. A Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) column in an isocratic mode with the mobile phase - acetonitrile: phosphate buffer (pH- 3.6) (50:50%v/v) was used. The flow rate was 0.8ml/ min and the detection was done at 252 nm. The retention time was found to be 2.850min. The linearity range was found to be 0.5 - 200 µg/ml. The present method is also useful for the estimation of valacyclovir in pharmaceutical dosage forms. The proposed method was subsequently validated. The method even proved to be affective on application to a stressed marketed formulation.

Key words: valacyclovir, RP-HPLC, stress studies, recovery studies.

INTRODUCTION

Valacyclovir is, L-Valine 2-[(2–amino–1, 6–dihydro–6–oxo–9H-purin–9yl) methoxy] ethyl ester[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)[2]. It is a prodrug to improve acyclovir oral bioavailability. Valacyclovir is rapidly converted to acyclovir, which inhibits DNA synthesis. Acyclovir is converted to the monophosphate by viral thymidine kinase, then to diphosphate by cellular guanylate kinase, and finally to the triphosphate by various cellular enzymes. Acyclovir triphosphate competitively inhibits viral DNA polymerase, and to a lesser extent human DNA polymerase[3]. Valacyclovir is available as tablet dosage form in the market. Few HPLC methods were reported for the determination of valacyclovir in pharmaceutical formulations[4,5], in biological fluids[6-10] and one spectrophotometric method was reported[11]. There were three stability indicating HPLC methods were developed for valaciclovir[12-14], but the reported methods were suffering with something or other disadvantage like high flow rate or not strictly following the ICH guidelines especially photo-stability studies. The proposed method is carried out strictly in accordance with ICH guidelines[15].

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Stress testing of the drug substance can help in identifying 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. Keeping into the view of susceptibility of valacyclovir under variety of conditions, it was felt that a HPLC method of analysis that separates the drug from the degradation products formed under ICH suggested conditions would be of general interest. These studies provide valuable information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies.

As on date no stability indicating liquid chromatographic method was reported in the literature for valacyclovir. Attempts were made to develop a suitable single stability indicating LC method that can be used to determine the related substances and also the assay of bulk samples of valacyclovir. This paper deals with the development of stability indicating analytical method using the samples generated from forced degradation studies.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20 µl injection of sample loop (Hemilton) (manual), and SPD 10A VP UV-Visible Detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1software. Hypersil ODS C_{18} (46 mm X 25 cm, 5mm) column was used for the separation. The pH of the solution was adjusted by using digital pH meter, Model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Valacyclovir is a gift sample obtained from Cipla Pvt. Ltd. (Vikroli, Mumbai, India). Commercial formulations were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India. Disodium hydrogen phosphate anhydrous, A.R. grade, was purchased from Merck Ltd., Mumbai, India.

Preparation of standard drug solutions

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

Chromatographic conditions

The mobile phase used in this study was a mixture of acetonitrile and phosphate buffer (pH- 3.6) in the ratio of 50:50% v/v. Stationary phase is Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) column. The contents of the mobile phase were filtered before use through a 0.45µ membrane. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.8 ml/min for 10 minutes. The column temperature was maintained at 23 $\pm 1^0$C. The eluate was monitored at 252nm using UV-detector. The retention time of the drug was found to be 2.850min.

Calibration of standards

Calibration standards were prepared by spiking working standard solutions into mobile phase contained in 5 mL volumetric flasks to yield concentrations of 0.5, 1, 2, 5, 10, 25, 50, 100, and 200 µg /mL. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Then, 20µl of each of standard solutions were injected into the HPLC system for six times to get the chromatograms. The retention time, peak area of the drug, were recorded. The linearity range was found to be 0.5-200 µg/ml. The data of the regression analysis was shown in table-1.

Recovery of valacyclovir in tablets

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of valacyclovir was extracted with acetonitrile and phosphate buffer (pH- 3.6) in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45µm filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in triplicate. Figure-1 represents the typical chromatogram of valacyclovir in tablet dosage forms. The assay results were shown in table-2.

Forced degradation studies

Forced degradation studies were performed for bulk drug and formulations to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 0.1N HCl), base hydrolysis (using 0.1N NaOH), and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of the proposed method to separate valacyclovir from its degradation products.

About 25mg of valacyclovir RS drug was accurately weighed and transferred in to 25ml volumetric flask and diluted up to the mark with mobile phase. Valacyclovir at a concentration of 0.5 mg/ ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

Acid & alkaline degradation

Forced degradation in acidic media was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 0.1N HCl to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 48 hrs and neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 10mcg/ml. Similarly, forced degradation in alkaline media was performed using 0.1N NaOH. The representative chromatograms were shown in figure-2 and figure-3.

Oxidative degradation

Oxidative degradation was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 3%w/v of hydrogen peroxide to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 48 hours. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 10mcg/ml. The representative chromatogram was shown in figure-4.

Photostability

Valacyclovir API, tablet powder and solutions of valacyclovir were prepared and exposed to light to determine the effects of light irradiation on the stability of valacyclovir in solution and in the solid state. Approximately 50 mg of valacyclovir API powder was spread on a glass dish in a layer that was less than 2mm thick and a solution of API (1 mg/ml) was prepared in mobile phase. Tablet powder was also prepared in the same way. All samples for photo-stability testing were placed in a light cabinet and exposed to light for 40 h resulting in an overall illumination of ≥210Wh/m^2 at 25 ◦C with UV radiation at 320–400 nm. Control samples, which were protected with aluminum foil, were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as previously described. The representative chromatogram was shown in figure-5.

RESULTS AND DISCUSSION

HPLC method development and optimization

The chromatographic method was optimized by changing various parameters, such as the mobile phase composition, PH of the buffer used in the mobile phase. Retention time and separation of peaks of valacyclovir were dependent on pH of the buffer and the percentage of acetonitrile. Different mobile phases were tried, but satisfactory separation, good symmetrical peaks were obtained with the mobile phases consisting of acetonitrile and phosphate buffer (pH- 3.6) in the ratio of 50:50% v/v.

Results of forced degradation studies

Forced degradation studies were performed for bulk drug and formulations to provide an indication of the stability indicating property and specificity of the proposed method. Degradation was not observed in valacyclovir samples under stress conditions like acid hydrolysis and oxidation. The drug degradation was observed when valacyclovir was exposed to UV light (overall illumination of ≥210Wh/m^2 at 25^{o}C for 40 hrs with UV radiation at 320–400 nm) and when treated with alkali (0.1N NaOH for 48 h at room temperature). The major impurity formed under photolytic stress conditions was un-known impurity. Under alkali hydrolysis (in 0.1N NaOH for 48 h stress at RT), one major and some minor unknown degradation products were formed. This degradation is mainly observed in terms of loss of assay. The degradation with an acid (Figure 2) and peroxide (Figure 4) didn't show any additional peak. The assay of valacyclovir was unaffected by the presence of degradants and impurities which confirms the stability-indicating power of the method.

Method validation

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, specificity, recovery and robustness/ruggedness.

Linearity

The standard curve was obtained in the concentration range of 0.5-200µg/mL. The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method. The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

Limit of detection (L.O.D) and Limit of quantification (L.O.Q)

Limit of detection was found to be 0.142 µg/mL (signal to noise ratio is 3) and Limit of quantification was found to be 0.473 µg/mL (signal to noise ratio is 10).

Precision and accuracy

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard solutions were made and the response factor of drug peaks and percentage coefficient of variance (C.V) were calculated. In the inter day variation studies, six repeated injections of standard solutions were made for three consecutive days and response factor of drug peaks and percentage C.V were calculated. From the data obtained, the developed RP-HPLC method was found to be precise. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate. The represented data was shown in table-3.

System suitability

For system suitability, six replicates of standard solution was injected and studied the parameters like theoretical plates, tailing factor (k), Height Equivalent Theoretical Plate (HETP), capacity factor (k^1), peak symmetry of samples. The represented data was shown in table-4.

Robustness

The percent recovery of valacyclovir was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for valacyclovir was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked and the results are reproducible under differences in reagents, analysts and experimental periods. Hence, the proposed method was found to be rugged.

Specificity

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 10 min. as shown in Figure-1. The figure shows that valacyclovir is clearly separated from other excipients of the formulation. The retention time, asymmetric factor and peak area ratio of the marketed formulations were not affected with excipients present in formulation. From the results of stress testing studies, the proposed method has the ability to separate the analyte from its degradation products, indicated a high degree of specificity of this method. The degradation product(s) of the parent compound was found to be similar for both the tablets and API powder assessed. Thus, the HPLC method presented in this study is selective and specific for valaciclovir.

CONCLUSION

In this paper, the simple, accurate and well-defined stability indicating HPLC method for the determination of valacyclovir in the presence of degradation products was described. The behavior of valacyclovir under various stress conditions were studied and presented. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies

ACKNOWLEDGEMENTS

The authors are thankful to the Roland Institute of Pharmaceutical Sciences, Berhampur-760010, Orissa, for providing the laboratory facilities.

Table-1: Regression analysis of the calibration curve for valacyclovir

Parameters	Values
Linearity ranges (mcg/ml)	0.5-200 mcg/mL
Slope	51307
Standard deviation of slope	48.383
Intercept	46133
Standard deviation of intercept	51.216
Correlation coefficient (r)	0.9998

Table - 2: Assay results of tablet formulations using proposed method

Formulation	Labeled amount (mg)	Amount recovered (mg)[a] Mean ±SD	% Recovery[a] Mean, %RSD
Valcivir (tablets)	500	501.377±1.025	100.275, 0.204

[a] mean value ± standard deviation of three determinations

Table-3: Summary of validation parameters for the proposed method

Parameters	Values
Accuracy (%)	99.08 – 99.81
Precision (RSD[b], %)	
Intra day (n=3)	0.45 –0.56
Inter day (n=3)	0.82 – 1.31
Repeatability (RSD[b], n=6)	0.16 – 0.78

[b] RSD indicates relative standard deviation

Table-4: System suitability test parameters for valacyclovir by the proposed method

Parameters	Values
Capacity factor	3.42
Theoretical plates	7392
Tailing factor	1.09
HETP	$5.8x10^{-5}$
Asymmetry	1.77

Table –5: Summary of forced degradation results

Stress condition	Time (hrs)	Mean Peak area[c]	% Recovery	Retention time of analyte	Retention time of major degradants
API	---	582534	100	2.842	---
Acid hydrolysis (0.1N HCl at RT)	48	573961	98.52833	2.842	1.967, 2.258
Base hydrolysis (0.1N NaOH at RT)	48	47643	8.178578	2.842	1.992, 2.242
Oxidation (3% H_2O_2 at RT)	48	572307	98.24439	2.842	1.950, 2.242
Photolysis (UV chamber)	40	544850	93.53102	2.842	1.967, 2.250

RT-room temperature; API- Active Pharmaceutical Ingredient; [c] mean peak area is the average of three determinations

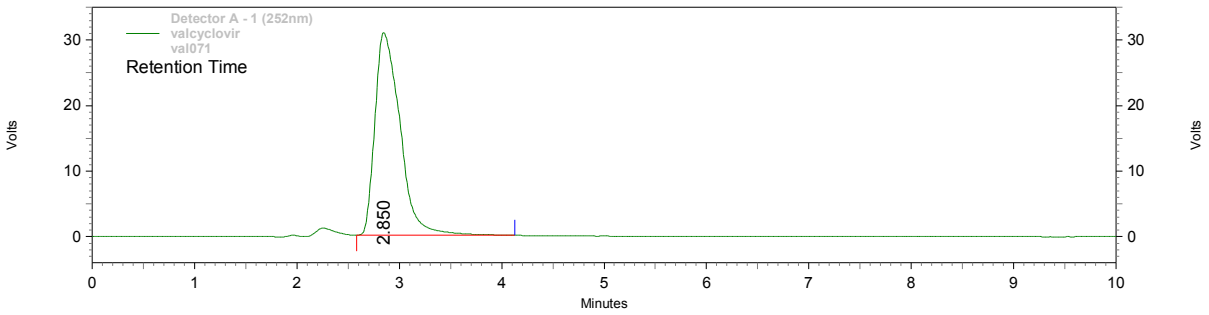

Fig-1: Typical chromatogram of valacyclovir in tablet dosage forms

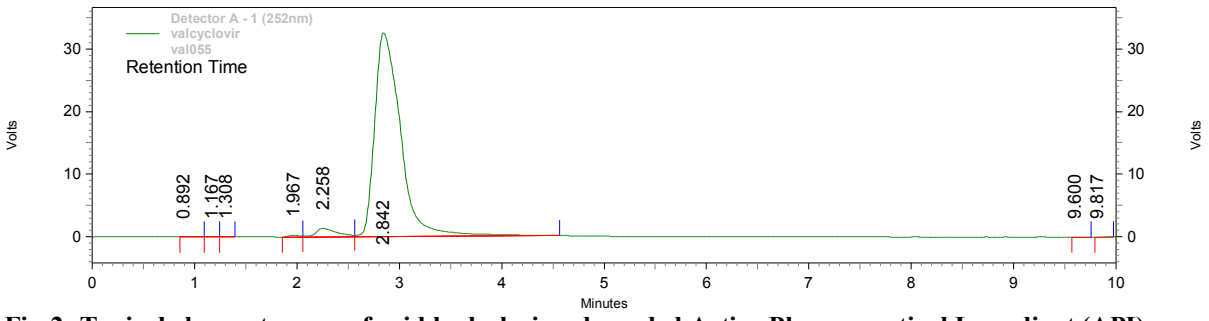

Fig-2: Typical chromatogram of acid hydrolysis – degraded Active Pharmaceutical Ingredient (API)

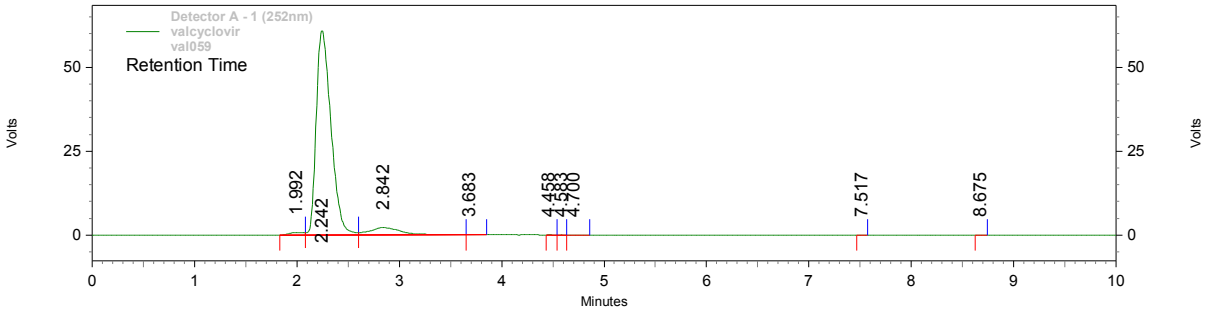

Fig-3: Typical chromatogram of alkaline hydrolysis – degraded API

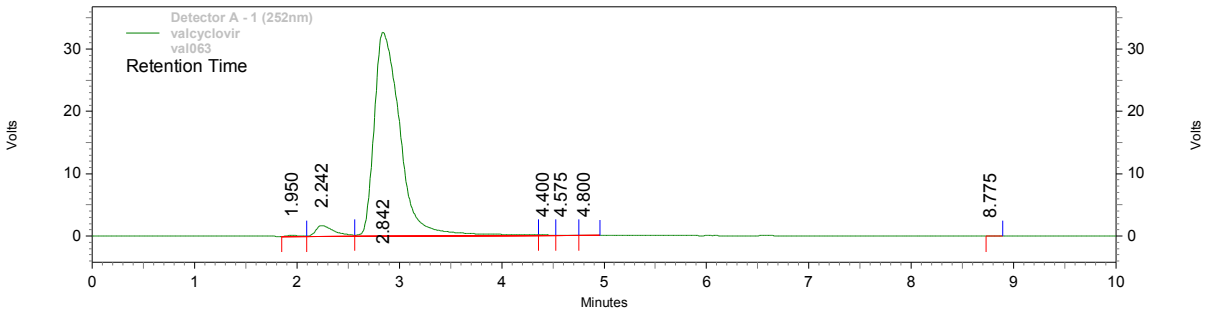

Fig-4: Typical chromatogram of oxidative hydrolysis – degraded API

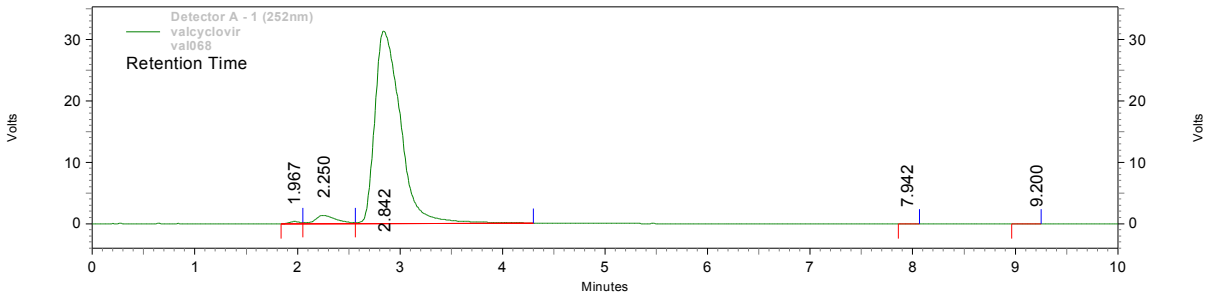

Fig-5: Typical chromatogram of Photolytic – degraded API

REFERENCES

1. Moffat C., Osselton M.D. and Widdop B., Clarks Analysis of Drugs and Poisons, 3rd ed. London, Pharmaceutical Press, 2004.
2. Martindale., The Complete Drug Reference, 33rd ed. London, Pharmaceutical Press, 1999.
3. Weller S. and Blum R. and Doucette M., Pharmacokinetics of the acyclovir pro-drug valacyclovir after escalating single- and multiple-dose administration to normal volunteers, Clin. Pharmacol. Ther., 1993;54:595—605.
4. Jadhava A.S., Patharea D.B. and Shingare M.S., Development and validation of enantioselective high performance liquid chromatographic method for valacyclovir, an antiviral drug in drug substance, J. Pharm. Biomed. Anal., 2007,43(4),1568-72.
5. Palacios M.L., Demasi G., Pizzorno M.T. and Segall A.I., Validation of an HPLC Method for the Determination of Valacyclovir in Pharmaceutical Dosage, J. Liq. Chromatogr. Rel.Technol., 2005, 28 (5), 751 – 62.
6. Maria K., Evagelos G., Sofia G., Michael K. and Irene P., Selective and rapid liquid chromatography/negative-ion electrospray ionization mass spectrometry method for the quantification of valacyclovir and its metabolite in human plasma, J.Chromatogr B 2008, 864(1-2), 78-86.
7. Ayhan S., Cansel K.Z., Yalin Z., Bengi U. and Sibel A.Z., Development and validation of an RP-HPLC method for the determination of valacyclovir

in tablets and human serum and its application to drug dissolution studies. J. Liq. Chromatogr. Rel. Technol., 2003, 26 (11), 1755-67.

8. Chuong P.H, Fotoula S., Pierre S., Jean-Michel S., Sophie P. and Catherine G., Rapid determination of valacyclovir and acyclovir in human biological fluids by high-performance liquid chromatography using isocratic elution., J. Chromatogr. B. Biomed. Sci. Appl., 1999, 732 (1), 47-53.
9. Raja Rajeswari K., Nageswararao P., Sankar G.G., Rao AL., Raju D.B. and Rao J.V.L.N.S., Estimation of valacyclovir in tablets and human serum by RP-HPLC method, Asian J Chem 2006;18:2515-18.
10. Jan L. and Clas M., Acyclovir levels in serum and cerebrospinal fluid after oral administration of valacyclovir. Antimicrob, Agents. Chemother., 2003, 47, 2438-41.
11. Srinu babu G., Sarat babu I., Kiran kumar N., Yugandhar N.M., Raju C.H.A.I., Spectrophotometric determination of valacyclovir in pharmaceutical formulation, Asian. J. Chem., 2007,19(2),1642-44.
12. Palacios M. L., Demasi G., Pizzorno M. T. and Segall A. I., Validation of an HPLC method for the determination of valaciclovir in pharmaceutical dosage, Journal of Liquid chromatography & related technologies., 2005, 28(5), 751-762
13. Patil1 G. D., Yeole P. G., Manisha P and Wadher S. J., 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, International Journal of Chem Tech Research., 2009,1(1), 16-26
14. Fish D.N., Vidaurri V.A and Deeter R.G., Stability of valacyclovir hydrochloride in extemporaneously prepared oral liquids, American Journal of Health-System Pharmacy., 1999, 56 (19), 1957-1960
15. ICH, Stability Testing of New Drug Substances and Products; International Conference on Harmonization., IFPMA, Geneva, 2003.

*****