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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

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Abstract

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. As per ICH guideline Q1A (R2), drug was subjected to all stress conditions such as hydrolysis (acidic, neutral and alkaline), oxidation (3 % H_2O_2 v/v), photolysis (As per ICH guideline Q1B), thermal degradation and humidity study. All stressed samples were successfully analysed on C_8 column using mobile phase Acetonitrile: Phosphate buffer pH 3 (25 mM) in the ratio of 10:90 v/v. A flow rate was maintained at 1 ml/min and detection was made at 254 nm. The drug was found to degrade extensively in alkaline, acidic and oxidative conditions, and also in the presence of light (in alkaline environment). Mild degradation was found in neutral but the drug was stable to thermal, humidity stress. The developed method was validated over the linearity, precision, accuracy, ruggedness and specificity as per ICH guideline Q2B. The major degradants was identified as acyclovir 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 in the presence of its degradation products in the bulk drug and tablets. It can be used for analysis of samples during stability testing.

Keywords: Valacyclovir; reversed phase liquid chromatography, stability indicating method, forced decomposition.

1. Introduction

Valacyclovir is, L-Valine 2-[(2–amino–1, 6–dihydro–6–oxo–9*H*-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] . Valacyclovir is available as tablet dosage form in the market. Few HPLC methods were reported for the determination of valacyclovir in its pharmaceutical formulation[3,4] and in serum[5] . Previously reported HPLC method using C_{18} with flow rate 3 ml/min, our reported liquid chromatographic method determined valacyclovir in presence of acyclovir using C_8 with flow rate 1 ml/min. The ICH guideline Q1A (R2) 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 Q1 (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[6]. 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[7] .

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 no method is as yet reported for the determination of valacyclovir in the presence of its degradation product in bulk as well as tablet dosage form.

2. Experimental

2.1. Chemicals

Standard valacyclovir and acyclovir were gifted by Cipla Pvt. Ltd. (Vikroli, Mumbai, India). HPLC grade acetonitrile and water were procured from LOBA Chemie Pvt. Ltd (Mumbai, India). Acetic acid, potassium dihydrogen phosphate, di-sodium hydrogen phosphate, sodium hydroxide, hydrogen peroxide and all other chemicals of analytical reagent grade were procured from LOBA Chemie Pvt. Ltd (Mumbai, India).

2.2. Instrumentation

Analysis of all stressed samples were performed using JASCO PU 1575 HPLC system consisted of a 1580 intelligent pump, a 1575 intelligent UV- Visible detector with precision loop injector (Rhenodyne, 20 µl). The data was processed by using BORWIN 1.27 software. All samples were filtered through 0.45 µm membrane Millipore filtration apparatus with vacuum pump.

2.3. Chromatographic conditions

Stability indicating HPLC assay method was developed on Jasco intersil C_8, 250 x 4.6 mm, 10 µm column, using a mobile phase containing acetonitrile: 25 mM phosphate buffer pH 3 (10: 90, v/v) at ambient temperature. The flow rate was maintained 1 ml/ min throughout analysis. Initially the method was developed for standard valacyclovir then it was extended to stress samples. The standard and all stress samples were prepared in mobile phase.

2.4. Forced degradation studies (stress testing)

The drug concentration for all stress studies was taken 1 mg/ml as per standard literature. The bulk drug was subjected to hydrolysis by refluxing the drug solution in 0.01 N NaOH for 8 h. Similarly the acidic hydrolysis was performed by refluxing solution of drug in 0.1 N HCl for 8 h. The neutral hydrolysis was done in water at refluxing temperature for 8 h. The samples from all hydrolysis studies were withdrawn at an interval of each 2 h just to check the time being degradation of drug under stress conditions. All samples were taken in different 100 ml volumetric flasks and dissolved in mobile phase. Volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper No. 41 and injected in the chromatographic system[8] .

Oxidation studies were performed on bulk drug in 3 % H_2O_2 at ambient conditions for 7 days while, metal transition test was done using 25 mM copper in water at ambient conditions for 7 days.

Photostability test was performed as liquid state photostability study and solid state photostability study. In liquid state study pure drug was exposed to photo degradation test in 0.01 N NaOH, 0.1 N HCl and in water at ambient conditions for 15 days. Whereas in solid state study pure drug was sufficiently spread on petri plates (1 mm thick layer) and tablets also exposed to photo degradation test at ambient conditions for 2 months. At the same time controls of all samples were also exposed to same conditions in photostability chamber. As per ICH guideline Q1B (option 2) photostability test was performed[9] .Accurately weighed 50 mg of valacyclovir pure drug was dissolved in water, 0.1N HCl and 0.01 N NaOH separately in 100 ml volumetric flask. All the solutions were kept

inside the photostability chamber at ambient condition. The samples were withdrawn at 0, 8, 15 days interval and further dilution with mobile phase to get concentration 40 µg/ml and each sample was analyzed by injecting in chromatographic system.

Bulk drug and tablet dosage form were subjected to dry heat at 50 ^{0}C for 3 months[10].

Humidity study was performed separately to accelerated conditions at 40^{0}C± 2^{0}C and 75 %RH ± 5% RH for 3 months in the stability chamber. All stressed sample were withdrawn periodically and analyzed by developed HPLC method. The % residual drug to be remained was calculated from the standard calibration curve.

2.5. Method validation

Validation of developed analytical method was performed as per ICH guideline Q2 B[11],over the linearity, accuracy, precision, specificity, limit of detection, limit of quantitation and robustness.

3. Results and discussion

3.1. Analysis of stressed samples

The ICH stability guideline Q1A (R2) defines stress testing for new drug substances and drug products, to elucidate the intrinsic stability of the drug substances and drug products. The stress testing may also provide information about degradation pathways and selectivity of the applied analytical method.

Analysis of all stressed samples was performed using acetonitrile: phosphate buffer pH 3 (10:90 v/v) as the mobile phase.

It drug was found to degrade extensively on heating it in 0.1 N HCl for 8 h at reflux (Fig. 3) and in 0.01 N NaOH for 2 h at 25 ^{0}C (Fig. 4), while in neutral condition, only 8-9 % degradation (Fig. 5) was seen upon heating the drug at reflux for 8 h. It was observed that drug degraded (Fig. 6) around 10-40 % when exposed to oxidation (3 % H_2O_2 and 25 mM Copper powder). Also the drug degradation (Fig. 7) was observed when valacyclovir was exposed in the liquid state (0.1 N HCl, 0.01 N NaOH and Neutral) to Light as per ICH Guideline Q1B option 2, whereas slight degradation was seen in the solid state photostability study. But the drug was found to be stable when exposed to thermal study at 50 ^{0}C for 3 months and humidity study at 40 ^{0}C ± 2 / 75 % RH ± 5 % RH for 3 months. In all stressed samples 2 different degradation products were seen, one major degradation product was formed as acyclovir, which was identified by comparing with standard acyclovir. But in case of liquid state photostability in 0.01 N NaOH and neutral condition

one different degradation product found at retention time 5.81 min., which was not observed in other stress studies.

The developed method was extended to marketed tablets formulation but not seen a even single peak of degradation product when subjected to dry heat at 50^0C for 3 months and humidity study at 40 ^{0}C ± 2 / 75 % RH ± 5 % RH for 3 months.

3.2. Validation of the method

3.2.1. Precision

Precision of the proposed method was evaluated through intraday and Interday. The % R.S.D. was found to be <0.61 and <0.93, respectively, which met the acceptance criterion for the established method. The data is quoted in Table I.

Table I

Precision studies (n = 6)

Spiked concentration (µg/ml)	Measured concentration (µg/ml) ± S.D., % RSD	
	Intraday	Interday
4	3.96 ± 0.316, 0.452	3.92 ± 0.274, 0.301
6	5.99 ± 1.451, 0.511	5.95 ± 1.210, 0.785
8	8.35 ± 1.104, 0.873	8.17 ± 2.581, 0.563

3.2.2. Accuracy

Excellent recovery was obtained by fortifying three different concentration of valacyclovir standard in the mixture of decomposed sample obtained from all stress tests. The data is presented in Table II.

Table II

Recovery study (n = 3)

Spiked concentration (µg/ml)	Measured concentration (µg/ml) ± S.D., % RSD	% Recovery
4	3.92 ± 1.530, 0.741	98.0
6	5.91 ± 1.296, 0.385	98.5
8	7.95 ± 0.659, 0.241	99.4

3.2.3. Linearity

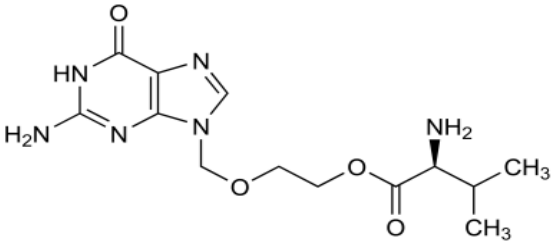
The linearity was studied for valacyclovir. An accurately weighed quantity of valacyclovir (100 mg) was dissolved in mobile phase and volume was made up to 100 ml with mobile phase (1000 µg/ml). The aliquot portions of standard stock solution were mixed and diluted appropriately with mobile phase to get the series of concentration covering ranges from 20-400 µg/ml for valacyclovir. The method was found to be linear in the studied range. The linear regression equations of valacyclovir was y = 35113x with r = 0.998 where 'x' was the concentration of standard, 'y' was the observed response and 'r' was the correlation coefficient.

3.2.4. Limit of detection (LOD) and limit of quantitation (LOQ)

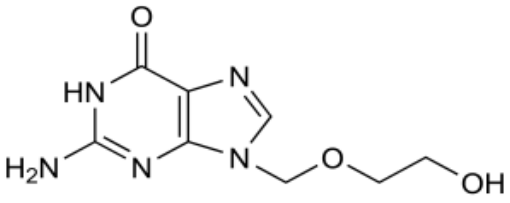
LOD and LOQ were determined from standard deviation and slope method as per ICH guideline, for valacyclovir LOD was found to be 0.0048 µg/ml and LOQ was found to be 0.0144 µg/ml.

3.2.5. Specificity

Good separation of valacyclovir and its degradation products were from each other. A resolution factor for the drug peak was 4.5 from the nearest resolving peak with no interference from the sample matrix proved that the method was found to be specific to the drug.



Valacyclovir



Acyclovir

Fig. 1: Structure of valacyclovir and its major degradation product acyclovir

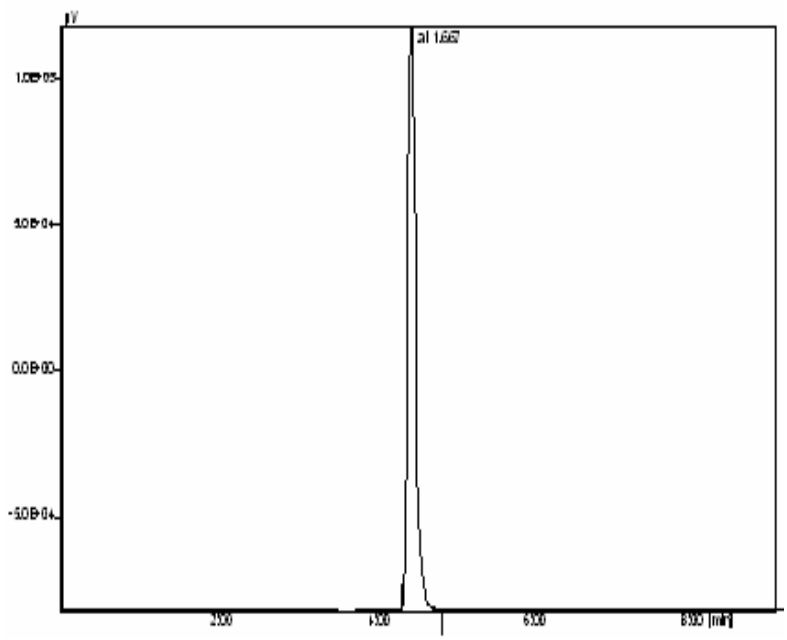


Fig. 2: HPLC chromatograms representing standard solution of valacyclovir

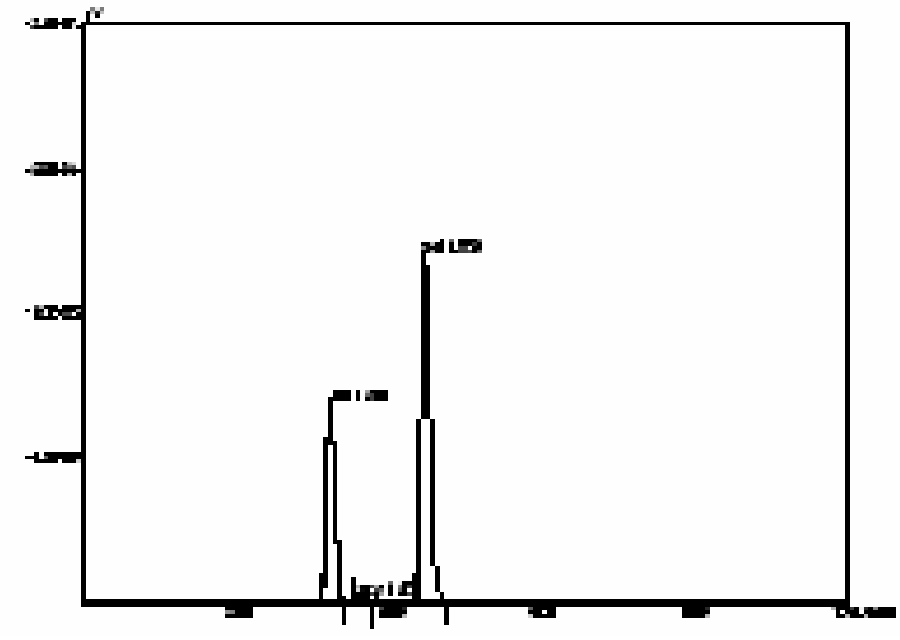


Fig. 3: HPLC chromatograms representing degradation behavior of valacyclovir in 0.1 N HCl

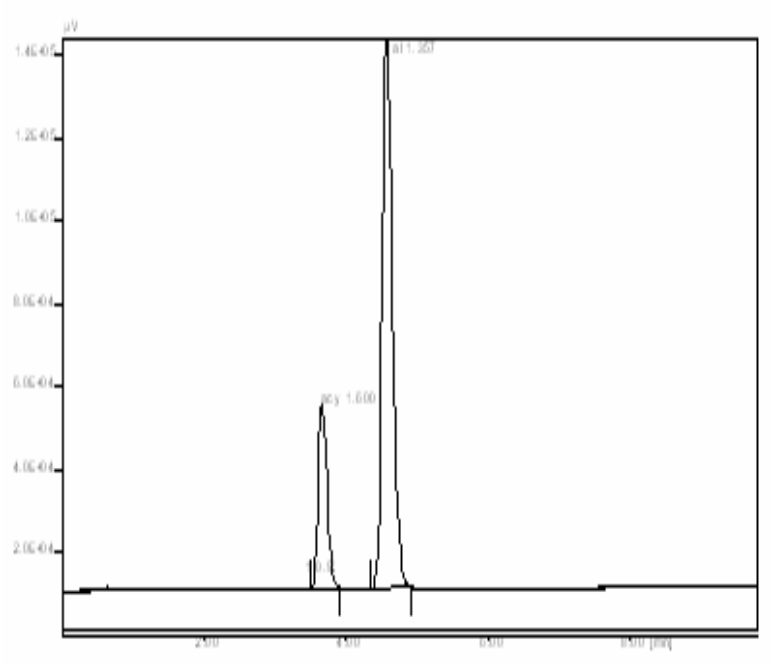


Fig. 4: HPLC chromatograms representing degradation behavior of valacyclovir in 0.01 N NaOH

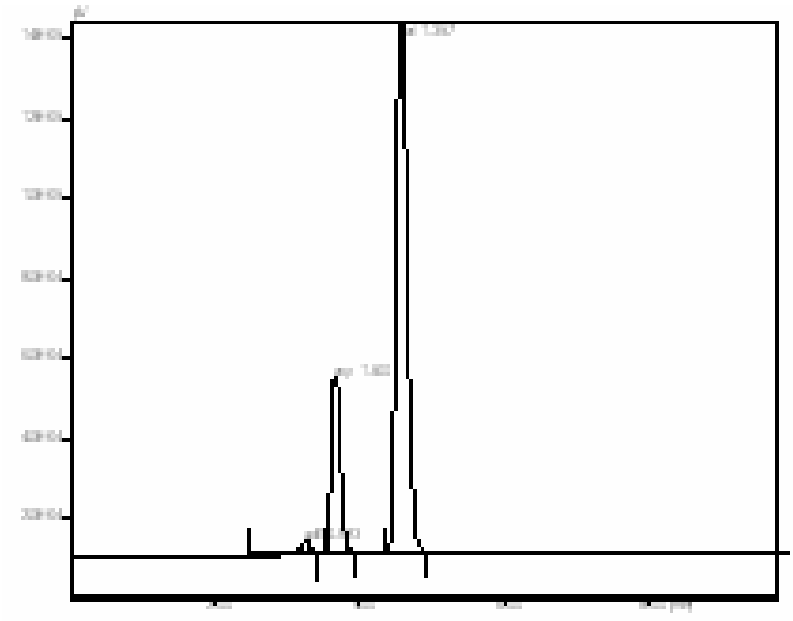


Fig. 5: HPLC chromatograms representing degradation behavior of valacyclovir in water

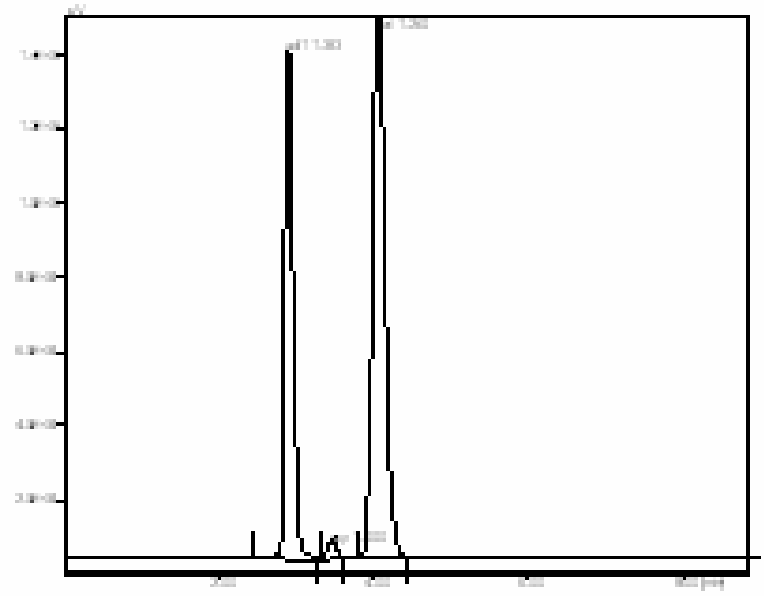


Fig. 6: HPLC chromatograms representing degradation behavior of valacyclovir in 3 % H_2O_2

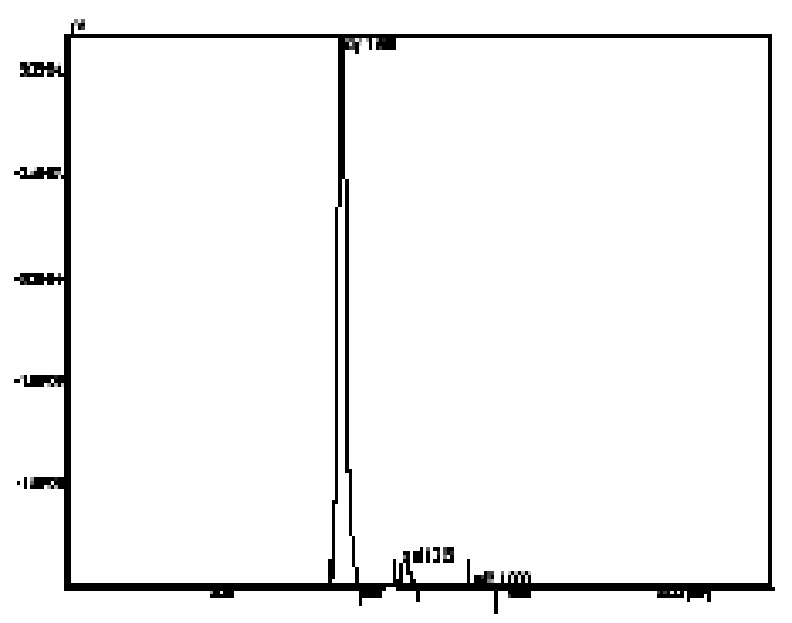


Fig.7: HPLC chromatograms representing degradation behavior of valacyclovir in liquid state photostability in 0.01 N NaOH.

Conclusion

The study brings forward new and interesting aspects on the decomposition behaviour of valacyclovir. A stability indicating reversed phase liquid chromatograph 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.

The proposed 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 solvent consumption along with the short analytical run time of 10 min leads to cost effective chromatographic method.

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