Research Article

Development and Validation of RP-HPLC Method for the Determination of Valacyclovir Hydrochloride and its Related Substances in Tablet Formulation

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Accepted on: 13-12-2013; **Finalized on:** 28-02-2014.

ABSTRACT

A chiral high performance liquid chromatographic method was developed and validated for the separation of Valacyclovir drug substance and its related substances V1 (guanine), V2 (acyclovir) and V3 unknown impurity). The Valacyclovir and its impurities were resolved on 150 x 4.0 mm (i.d.), stainless steel, packed with 5µm Daicel Chiral Phase Crownpack CR (+) column at 15°C using 0.1% aqueous Phosphoric acid (85%): Methanol (90:10 V/V) as a mobile phase. A PDA detector set at 254 nm was used for detection. The linearity for related substances was obtained within concentrations ranging from 0.3 to 6µg/mL. The correlation coefficient of Valacyclovir was 0.9997. Relative standard deviations of peak areas from six measurements were always less than 2%. TThe proposed method was found to be suitable and accurate for the quantitative determination of Valacyclovir in bulk drug substance and tablet formulation. Validation parameters showed that the method is specific, accurate, precise and reproducible. The method can be used for routine quality control and stability analysis of Valacyclovir drug substance.

Keywords: HPLC, Related substances, Valacyclovir, Validation.

INTRODUCTION

Valaciclovir hydrochloride (VAL) [(*S*)-2-[(2-amino-6-oxo-6,9-dihydro-3*H*-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate] (Figure 1) is a hydrochloride salt of L-Valyl ester of acyclovir.[1-3] It is an oral antiviral drug used to treat infections with herpes zoster (shingles), herpes simplex genitalis (genital herpes), and herpes labialis (cold sores). It inhibits the replication of viral DNA. It is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir is converted by esterase to active drug acyclovir via hepatic first pass metabolism.[3]

Literature survey revealed that few Spectrophotometric methods[4-11], HPLC methods[12-18], and LC-MS methods for biological fluids[19-23] are reported in the literature for the determination of VAL in Bulk, pharmaceutical formulations and serum samples.

The aim of the present work was to develop a simple and economic liquid chromatographic method that would be suitable for determination of VAL and its impurities in bulk and dosage form. The proposed method is found to be simple, accurate, reproducible and suitable for routine determination of VAL from its pharmaceutical dosage form.

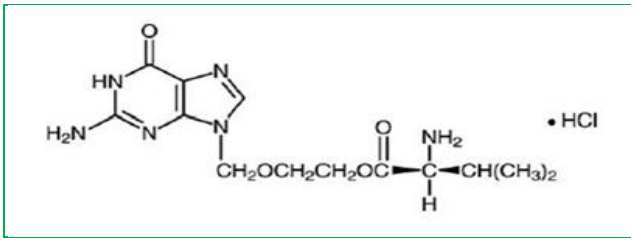

Figure 1: Structure of Valacyclovir Hydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Valacyclovir Hydrochloride (VAL) reference standard, Valacyclovir bulk drug and related substances were procured from GlaxoSmithkline Nashik (India). Methanol used was of HPLC grade (Qualigens, Mumbai). Valcivir tablets 500mg (Cipla Pharmaceutical Ltd., Goa, India) were used for the assay.

Equipments

HPLC system : Water's HPLC system

Column : 150 x 4.0mm (i.d.), Stainless steel, packed with 5µm Daicel Chiral Phase Crownpack CR (+)

Balance : Mettler Toledo 205

Ultrasonicator : ENERTECH Electronics Pvt. Ltd.

Preparation of solutions

Standard Stock solution

Standard stock solution containing 1000 µg/mL of the VAL was prepared in 0.1N hydrochloric acid using VAL reference standard.

Diluent

A 0.1% v/v aqueous phosphoric acid (85%) was used as a diluent.

Stock solutions of related substances

The separate stock solutions of related substances V1 (guanine), V2 (acyclovir) and V3 of concentrations 100µg/mL were prepared by dissolving 10mg of each

related substance in diluent separately and diluting to 100mL with the same solvent.

Resolution Check Solution

The solution containing 50µg/mL of VAL reference standard and 0.8µg/mL of related substance V3 was prepared in diluent. The resolution between peaks due to V3 and VAL is not less than 1.5.

Chromatographic Conditions

Analysis was carried out on 150 x 4.0mm (i.d.), stainless steel, packed with 5µm Daicel Chiral Phase Crownpack CR (+) column using 0.1% v/v aqueous Phosphoric acid (85%): Methanol (90:10 V/V) as mobile phase at a flow rate of 0.8mL per minute. The column temperature was set at 15°C. The volume injected was 10µl and detection was carried out at 254 nm using a PDA detector.

Calibration curve

From the standard stock solution of VAL aliquots, (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mL) were transferred to series of 10 mL volumetric flasks and volume was made up to the mark with diluents to give solutions of concentrations in the range of 25-500 µg/mL. The chromatograms and peak areas of these solutions were measured at 254 nm and a calibration curve was constructed, by plotting the area against the corresponding drug concentration.

Related Substances by HPLC

A solution containing 100µg/mL of VAL and 0. 8µg/mL of Guanine (V1), acyclovir (V2) and V3 was prepared in diluent and injected in HPLC system. The retention time for each substance was recorded. Figure 2 shows the resolution of VAL and related substances.

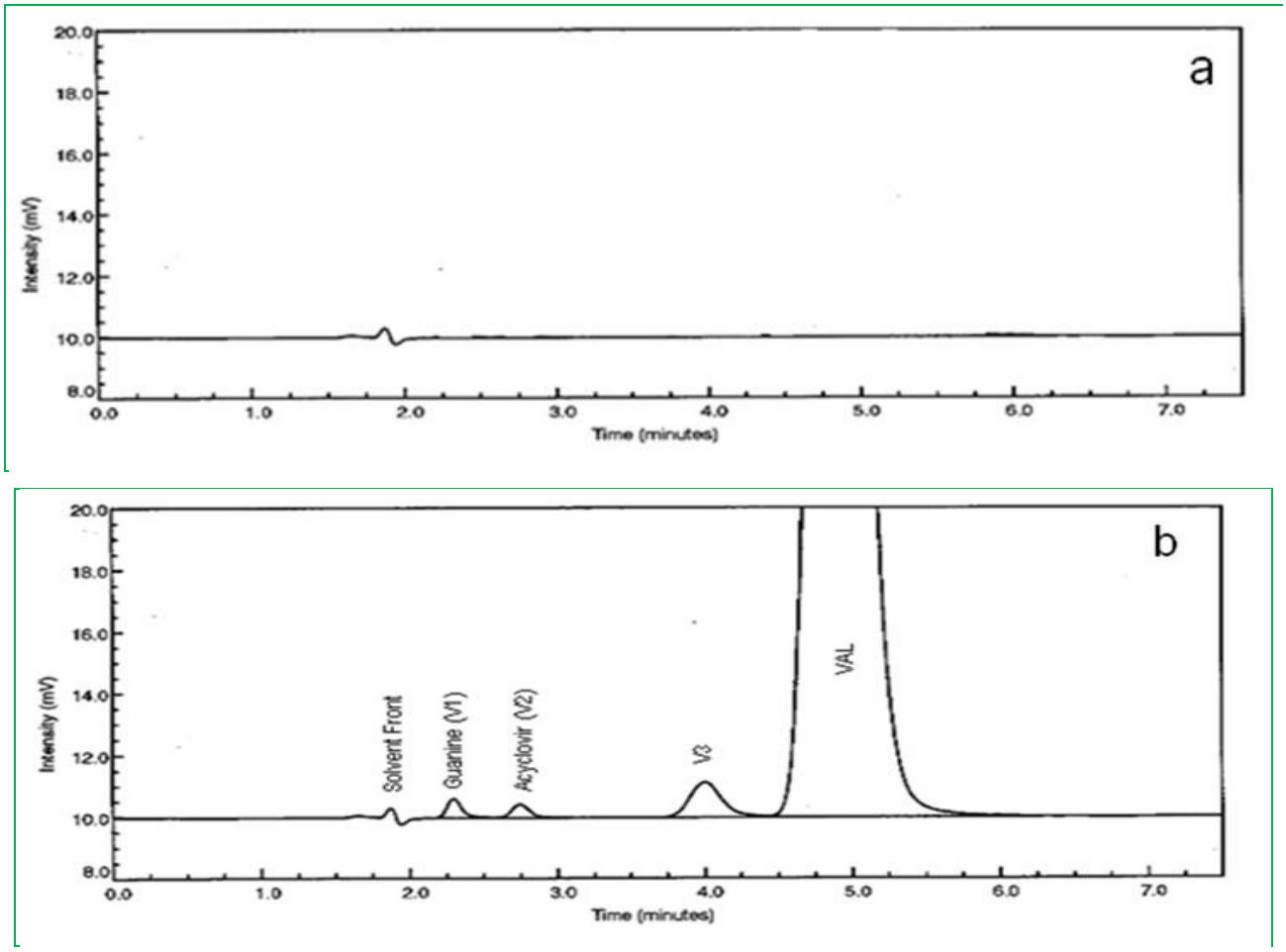

Figure 2: Typical chromatogram of a) mobile phase (Blank) and b) Standard VAL and related substances V1, V2, V3

Validation of the Method[25]

Linearity

The linearity of peak areas versus different concentrations was evaluated for VAL over the range of 25-500 µg/mL and for all the related substances over the range of 0.3µg/mL to 6µg/mL. The correlation coefficient (r^2) for VAL and each related substances was calculated**.**

Specificity

The analyte should have no interference from other extraneous components and be well resolved from them. To determine the specificity of the method, the mixture of reference standard VAL and the related substances was injected and chromatogram was recorded. The sample solution (pharmaceutical dosage form) was then injected and the chromatogram was obtained. The sample chromatogram was compared with the standard chromatogram.

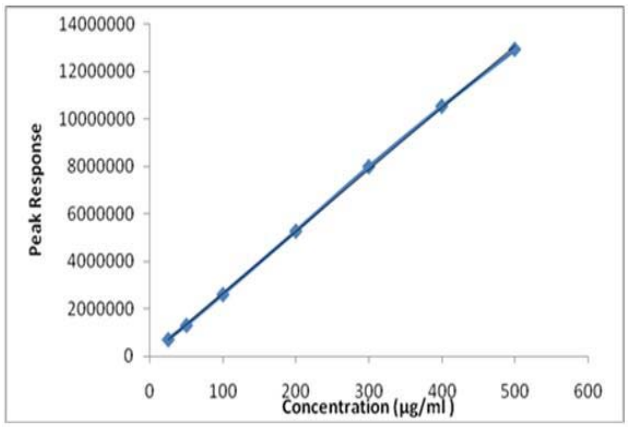

Figure 3: Linearity plot of standard VAL

Precision

Precision of the method was studied in terms of repeatability and intermediate precision.

Repeatability

Repeatability was performed by analyzing six separate VAL solutions of concentration 50 µg/mL that were prepared by spiking the related substances to give 1 µg/mL of each of V1, V2 and V3. The %R.S.D for each related substance was evaluated.

Intermediate precision

The intermediate precision of the method for VAL and related substances was determined on three separate sample solutions prepared by spiking the related substances by two different analysts on two different days. The mean values of results for each day and for each analyst were compared.

Accuracy (Recovery studies)

To ensure the accuracy of method, recovery studies were performed by standard addition method at 80 %, 100 % and 120 % levels of drug concentrations, to the pre-analyzed samples and they were re-analyzed.

Accuracy of the method for all the related substances was determined by analyzing VAL sample solutions spiked with all the related substances at three different concentration levels of 1%, 2% and 4% of sample concentration each in triplicate.

Application of the method for estimation of VAL in bulk drug

The bulk drug VAL was dissolved in 0.1N hydrochloric acid and diluted to give the solution containing 50 µg/mL of the VAL. The solution was injected and peak area was recorded.

Assay of VAL in tablets

Twenty tablets were accurately weighed, their average weight was determined and they were finely powdered. The powder equivalent to 50 mg of VAL was transferred to 100 mL volumetric flask and 50mL of 0.1N hydrochloric acid was added. The solution was sonicated for 10 minutes and volume was made up to the mark with 0.1N hydrochloric acid. The solution was filtered through 0.45µm filter and 1 mL of filtrate was diluted to 10 mL with diluent to give the solution containing 50µg/mL of the VAL. The solution was injected and peak area was recorded.

System Suitability Parameters

System suitability test was performed to verify that the resolution and reproducibility of the chromatographic systems were adequate for the analysis. Five injections of the standard were injected for this purpose. The retention time, areas, theoretical plates, peak asymmetry and resolution were calculated for standard solutions.

RESULTS AND DISCUSSION

A new HPLC method was developed for the determination of Valacyclovir related substances in Valacyclovir drug substance using 0.1%aqueous Phosphoric acid (85%): Methanol (90:10 V/V) as a mobile phase. Three related substances were detected by this method. The developed method was validated as per the ICH guidelines and was applied to estimate the drug from tablet formulations. The details of findings are as below.

Related Substances by HPLC

Three related substances were detected and well resolved by the method. The retention data for VAL and related substances is indicated in Table 1.

Table 1: Typical Retention Data

Component	Retention time (minutes)	Relative retention time (RRT)
Guanine (V1)	2.2	0.49
Acyclovir(V2)	2.8	0.57
V3	3.9	0.80
VAL	4.9	1.0

Linearity

The linearity for VAL was obtained in the concentration range of 25-500 µg/mL and for the related substances in the concentration range of 0.3 - 6 µg/mL. Linearity data for VAL and Related substances is summarized in Table 2.

Table 2: Linearity data for VAL and Related substances

Parameter	VAL	V1	V2	V3
Linearity range (µg/mL)	25-500	0.3-6	0.3-6	0.5-6
Slope	26078	20113	20027	12363
Y-intercept	46889	1556	813	867
Correlation coefficient (r^2)	0.9997	0.9999	1	0.9999

Specificity

Specificity of the method was demonstrated by no interference of related substances with the VAL peak. All the related substances were separated with good

resolution by the mobile phase developed for the method. Specificity of peak of VAL from tablet was determined by comparing with the peak of reference standard. No interference of excipients with the VAL peak was observed.

Precision

Precision of the method was studied in terms of repeatability and intermediate precision. Precision was expressed in terms of % R.S.D. All values for precision were within recommended limits.

Repeatability

Analysis of six separate solutions of VAL with concentration 50 µg/mL spiked with the related substances to give 1 µg/mL of each of V1, V2 and V3 showed the repeatability of the method. Satisfactory agreement of VAL retention times and peak area counts was observed as given in Table 3.

Table 3: Repeatability of VAL and Related substances

	Concentration found (µg/mL) Mean ± SD (n=6)	% RSD
VAL	48.12 ± 0.036	0.07
V1	1.02 ± 0.003	0.284
V2	1.02 ± 0.006	0.626
V3	0.999 ± 0.006	0.588

n=6: Mean of six determinations

Intermediate precision

Comparisons of the mean values for each day and for each analyst as summarized in Table 4 indicated that the method is precise and reproducible.

Table 4: Results of intermediate precision

Component	Concentration found (µg/mL) Mean ± %RSD, n=3			
	Analyst 1		Analyst 2	
	Day 1	Day 2	Day 1	Day 2
VAL	49.98 ± 0.14	49.98 ± 0.19	50.02 ± 0.09	50.00 ± 0.07
V1	1.02 ± 0.461	1.03 ± 0.386	1.02 ± 0.536	1.03 ± 0.414
V2	1.02 ± 0.480	1.02 ± 0.688	1.02 ± 0.660	1.02 ± 0.506
V3	0.99 ± 0.770	1.00 ± 1.070	1.00 ± 0.542	1.00 ± 0.780

n=3: Mean of three determinations

Accuracy

The results of recovery studies showed the accuracy of the method. The recoveries for VAL and related substances were ranged between 100.11 – 101.15 % and 98.43 – 101.77 %, respectively. Results obtained for VAL and related substances are given in Table 5 and Table 6 respectively.

Table 5: Recovery of VAL

Level	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery* (Mean ± SD)
80%	40	40.14	100.34 ± 0.39
100%	50	50.05	100.11 ± 0.14
120%	60	60.69	101.15 ± 0.07

*-Mean of three determinations

Table 6: Recovery of Related substances

Amount added (µg/mL)	V1		V2		V3	
	Amount found (µg/mL)	%Recovery ± SD *	Amount found (µg/mL)	%Recovery ± SD*	Amount found (µg/mL)	%Recovery ± SD*
0.5	0.502	100.31 ± 1.0	0.499	99.79 ± 0.40	0.492	98.43 ± 0.89
1	1.018	101.77 ± 0.12	1.015	101.52 ± 0.50	0.986	98.65 ± 0.17
2	2.02	100.85 ± 0.16	2.013	100.67 ± 0.13	2.012	100.59 ± 0.41

***** - Mean of three determinations

Assay of VAL in Bulk drug

The solution of bulk drug containing 50 µg/mL of the VAL was analyzed. The results of assay are as given in Table 7.

Table 7: Results of assay in Bulk drug

Concentration taken (µg/mL)	50
Concentration found (µg/mL), mean ± SD	50.01± 0.03
% RSD (n=6)	0.05

Assay of VAL in tablets

The concentration of tablet solution was determined using linear regression equation (using slope and Y-intercept) and amount of drug in tablet was determined. The results of assay in tablets are summarized in Table 8.

System Suitability Parameters

System suitability parameters were tested for the chromatographic conditions and results are as shown in Table 9.

Table 8: Results of assay in tablets

Labeled claim (mg)	500
Amount found (mg)	499.62
% labeled claim	100.61
% RSD (n=6)	1.05

Table 9: System Suitability Parameters

Parameter	Average	% RSD
Retention time	4.91	0.23
Peak Area	1351591	0.05
HETP	7691	
Tailing Factor	1.20	
Resolution	1.4	

CONCLUSION

The results of the validation tests indicate that the method is simple, accurate, precise and specific. This method is suitable for the routine quality control of the tablet dosage form.

Acknowledgements: The authors are very thankful to GlaxoSmithKline Pharmaceutical Ltd., Nashik, India for providing samples for this research work and management of Amrutvahini Sheti and Shikshan Vikas Sanstha and Amrutvahini College of Pharmacy, Sangamner, M.S., India for providing the facilities for the research work.

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Source of Support: Nil, **Conflict of Interest:** None.