

RP-HPLC METHOD FOR THE ESTIMATION OF VALACYCLOVIR IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, specific, accurate reverse phase liquid chromatographic method was developed for the estimation of Valacyclovir (VAL) in bulk and tablet dosage forms. A C₁₈ reverse phase column (Develosil) of 250×4.6mm dimensions and 5 μm particle size with mobile phase containing 0.1% v/v Formic acid: Acetonitrile (90:10 v/v) was used and eluents were monitored at 252 nm. The retention time of VAL was 3.98 min and showed a good linearity in a range of 10-50 μg/mL. The percent assay was 98.9% and mean percentage recovery was found to be 98.02%. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of VAL in tablet formulations.

Keywords: Valacyclovir, Develosil column, Reverse phase liquid chromatography, Validation.

INTRODUCTION

VAL¹ (Fig 1) is, L-valyl ester (9-[(2-hydroxy ethoxy) methyl] guanine hydrochloride), of acyclovir, after oral administration is rapidly converted into acyclovir which shows antiviral activity against herpes simplex virus type I (HSV-1) and (HSV-2), Varicella Zoster Virus (VZV). Acyclovir oral bioavailability was increased when administered in the form of VAL² and VAL rapidly converted to Acyclovir in vivo which inhibits DNA synthesis. VAL is available as tablet dosage form in market and few HPLC³⁻⁵ methods were reported for the estimation of VAL in pharmaceutical formulations and in biological fluids⁶ and one of spectrophotometric⁷ method were also reported. There are two stability indicating HPLC^{8, 9} methods were developed for VAL, but the reported methods were having disadvantages like high flow rate and high retention time and more organic phase and the aqueous phase is not compatible to LC-MS analysis. The proposed HPLC method utilizes economical solvent system as compared with the previous reported methods and is compatible with LC-MS analysis. The proposed HPLC method leads to better retention time, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of VAL in bulk drug samples and in pharmaceutical dosage forms.

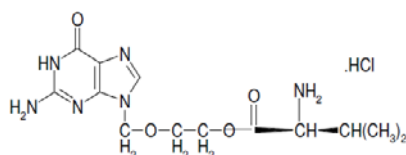


Fig. 1: Structure of VAL

MATERIALS AND METHODS

Reagents and Chemicals

VAL was a gift sample from Cipla Las, Ahmadabad, India. Acetonitrile, water and Formic acid were purchased from E. Merck, Mumbai, India. All the solvents and reagents are of HPLC grade. Val tablets of VALCIVIR® 500mg (B. No.A02469) were commercially purchased.

Equipment

Chromatographic separation was performed on a Shimadzu HPLC system equipped with a LC 10 AVP pump, M20 A PDA detector and LC 10ATVP auto sampler was used with 200 μL loop volume. LC solution software was used to collect and process the data.

Chromatographic Conditions

Mobile phase consisting of 0.1% v/v Formic acid: Acetonitrile (90:10%) at 1mL/min flow rate was used and the mobile phase was

filtered through membrane filter (Millipore Nylon disc filter of 0.45μm) and sonicated for 5 min in ultrasonic bath. For quantitative analytical purpose wavelength was set at 252 nm and the column, Develosil (250mm x 4.6mm) was used at ambient temperature. Injection volume was 20 μL.

Preparation of standard stock solution

Standard stock solutions of VAL of strength 1mg/mL were prepared using 0.1%v/v Formic acid. Appropriate amounts of these stock solutions were then further diluted with 0.1% Formic acid to get the required concentrations of standard stock solutions at a concentration range of 10-50 μg/mL.

VALIDATION

Linearity

The linearity of VAL responses at concentrations in the range of 10 to 50μg/mL was determined by preparing and injecting standard solutions (20μL). The data was given in Table 1.

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (30μg/mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (30μg/mL for sample application). The data was given in Table 2.

Accuracy

Accuracy (Recovery) of the method was tested by spiking 80, 100 and 120% of VAL working standard. The accuracy of the analytical method was established in triplicate across its range according to the assay procedure and the data was given in Table 3.

Robustness

Robustness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as mobile phase composition, flow rate, instrument and analyst. The data was given in Table 4.

LOD and LOQ

LOD and LOQ were determined from standard deviation and slope method as per ICH guidelines¹⁰.

Assay of VAL in pharmaceutical formulations

Twenty tablets were weighed and finely powdered and a powder quantity equivalent to 25 mg VAL was accurately weighed and transferred to a 25 mL volumetric flask and 10 ml of diluent (0.1%

formic acid) was added to the same. The flask was sonicated for 5 min and volume was made up to the mark with diluent. The above solution was filtered using Nylon disposable Syringe Filter (13 mm, 0.45 µm) and the filtrate was diluted with diluent so as to obtain a concentration in the range of 10-50 µg/mL as discussed above. The amount present in the each tablet was calculated by comparing the area of standard VAL and tablet sample. The data was given in Table 7.

RESULTS AND DISCUSSIONS

Acyclovir oral bioavailability was increased when administered in the form of VAL (which is a L- Valine ester prodrug)^{1,2}. VAL is rapidly converted to Acyclovir, in vivo, which inhibits DNA synthesis. Several HPLC - UV analytical methods were published for the estimation of VAL in bulk and pharmaceutical dosage forms and also to check the impurity profiles³⁻⁹, however they were not economical and used higher percentages of organic solvents and VAL retention times were longer. In the present investigation initial trials were made to develop LC conditions for the separation of VAL using 0.1% v/v of Formic acid (pH 2.5) as aqueous mobile phase and methanol and acetonitrile as organic modifiers. The 0.1% v/v of Formic acid was selected based on the fact that VAL was stable (without conversion to acyclovir) at below 4.0 pH fluids⁸ and also MS detector friendly nature for further transfer of this method to LC-MS analysis.

Initial trials were carried out with C₁₈ Phenomenex column (250 mmx4.6mm) using 0.01% v/v of Formic acid: Methanol (50:50) at a flow rate of 1.0 mL/min and the VAL peak was eluted before the solvent front at 2.47 min with tailing. In another trial using Methanol: Water (50:50) peak was eluted at 2.7 min, however, peak splitting was observed. In another trial Acetonitrile: Water (50:50)

peak was eluted at 2.1 min and peak obtained was broad and before the solvent front. Whereas, with the same mobile phase composition using Develosil column, peak was eluted at 2.4 min and peak splitting was observed. Further trials were carried out with 0.1% v/v Formic acid: Acetonitrile (50:50%) at 1mL/min flow rate and the peak was eluted at 3.2 min with good peak shape and symmetry and is closer to solvent front.

Finally, with 90:10 % composition peak was eluted at 3.9 min and peak shape and symmetry (asymmetric factor 1.33) was maintained. For quantitative analytical purpose wavelength was set at 252 nm, which provided better reproducibility with minimum or no interference. Under the above developed conditions (0.1% v/v Formic acid: Acetonitrile (90:10%) at 1mL/min flow rate) method was validated as per ICH guidelines¹⁰. A sample chromatogram along with UV spectrum and peak purity profile was shown in Fig 2. The peak purity index was found to be greater than > 0.9999 and is within limits.

A linear relationship was evaluated across the range (10-50 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (r²) was found to be 0.999 and shows good linearity. The data of the calibration curve was given in Table 1.

Precision studies were carried out in terms of repeatability. Six determinations of 100 % concentration at 30µg/mL level was evaluated and the data was given in Table 2 and shown Fig 3. The % RSD was found to be below 2 and fulfilled the ICH guidelines criteria.

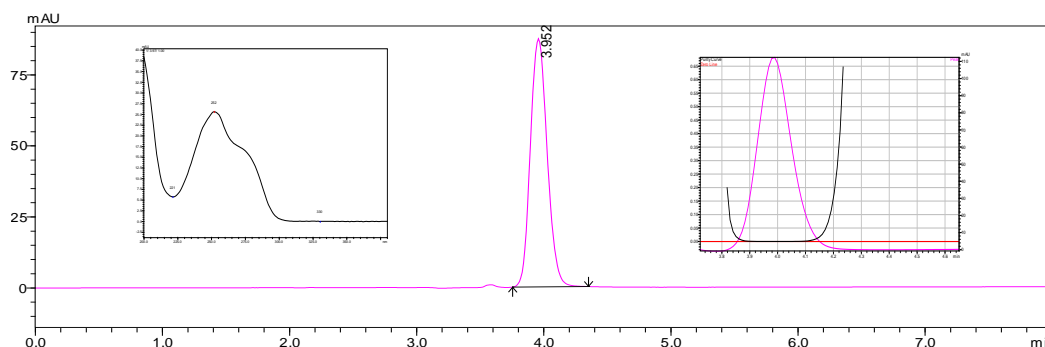


Fig. 2: VAL chromatogram with UV spectrum and peak purity profile and index*

(* Peak Purity Index:1.0000 ; Single Point Threshold :0.999447 ; Min.peak purity index:-552).

Table 1: Linearity data for VAL (n=3)

Concentration (µg/mL)	Peak Area (± sd)
10	210506 (54.00)
20	475274.7 (53.55)
30	697738 (147.52)
40	976144 (1829.13)
50	1206757 (6278.38)

y = 24934 x - 34727 r² 0.999

Table 2: Precision data of 30 µg/mL

Injection	Peak area
Injection-1	807331
Injection-2	804437
Injection-3	814621
Injection-4	791819
Injection-5	805975
Mean	806036
Standard Deviation (SD)	6030.311
% RSD	0.74%

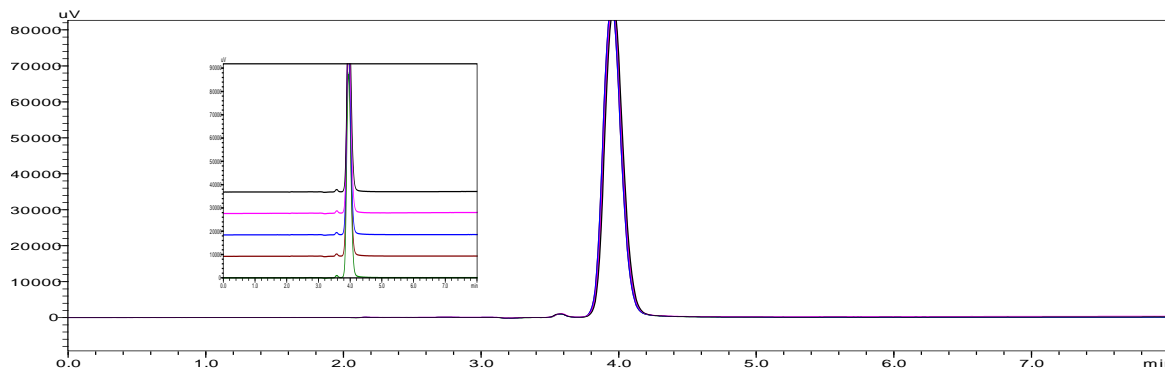


Fig. 3: Overlay of VAL 30 µg/mL chromatograms

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown and for drug substance the analyte peak is evaluated by 3D plot of the chromatogram in order to confirm the existence of one component at 3.9 min elution time of VAL (Fig 4) as

the impurities are not available. The recovery of the added standard to the drug product sample was calculated and it was found to be 97.70-101.5%, which indicates a good accuracy of the method to that of the labelled claim. The obtained recovery results were given in Table 3.

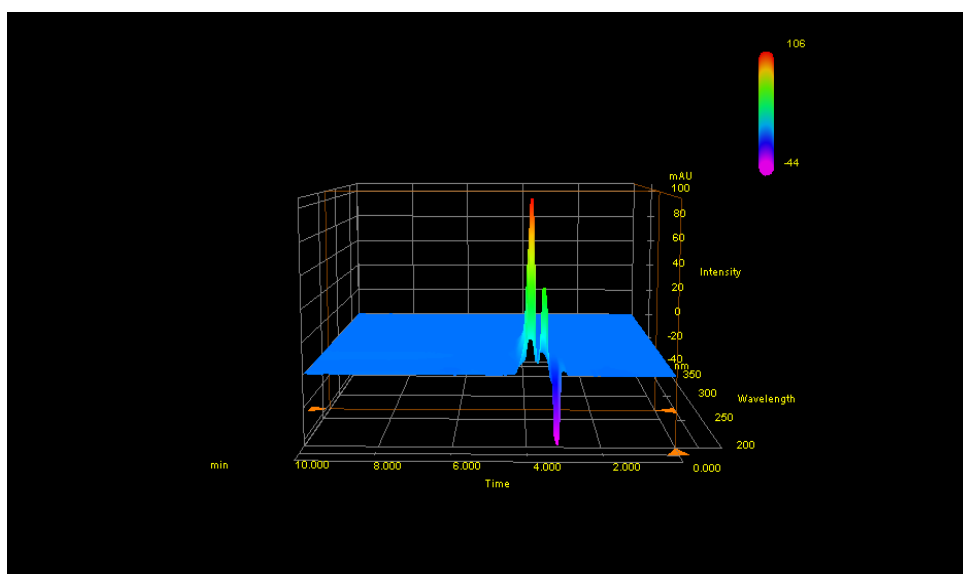


Fig. 4: 3D plot of the VAL chromatogram

Table 3: Accuracy data (n=3)

Level of recovery	Amount present (µg/mL)	Amount added (µg/mL)	% Recovery (Mean ± SD)	% RSD
80	30	24	97.93 ± 0.50	0.51
100	30	30	101.5 ± 0.10	0.10
120	30	36	97.70 ± 0.62	0.64

As a part of the robustness, deliberate changes in the flow rate, mobile phase composition, was made to evaluate the impact on the method. Retention times were significantly changed with flow rate and mobile phase compositions (Tables 4 and 5).

Table 4: Robustness data relating to flow rate change

S.No	Flow rate (mL/min)	Retention time (min)	Plate Count	Tailing factor
1	0.8	4.7	17156	1.6
2	1.0	3.9	17510	1.4
3	1.2	3.2	17179	1.6

Table 5: Robustness data relating to mobile phase composition change

S. No	Mobile phase	Retention time (min)	Plate Count	Tailing factor
1	2 % less	4.6	17065	1.7
2	Actual	3.9	17589	1.7
3	2 % more	3.5	17564	1.6

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD and LOQ were found to be 0.11µg/mL and 0.340 µg/mL respectively, indicating high sensitivity of the method.

System suitability was carried out by injecting 30 µg/mL of VAL at different injection volumes. The data was given in Table 6. With increment of injection volumes the RSD for tailing factor and theoretical plates number was less than 1% and is satisfactory.

The specificity of the method was established by spiking diluent solution of commonly used excipients in the form of tablet and

showed no peaks were observed at the retention time of VAL i.e 3.9 min and also over the range of 8.0 min as shown Figure 5.

The assay of Val in tablets was calculated by comparing the area of standard VAL and tablet sample along with the consideration of average tablet weight and weight of powder bled taken and dilution factor if any.

The assay was found to be within the limits (Table 7) and the present LC conditions can be used for the assay of VAL in different commercially available formulations.

Table 6: System suitability parameters

Injection volume (µL)	Retention time	Tailing factor	Theoretical plates
10	3.89	1.6	17963
20	3.91	1.4	17510
30	3.90	1.5	17449
40	3.88	1.6	17854
50	3.92	1.5	17179
Mean	3.9	1.52	17591
SD	0.015	0.083	317.81
%RSD	0.40	0.55	0.85

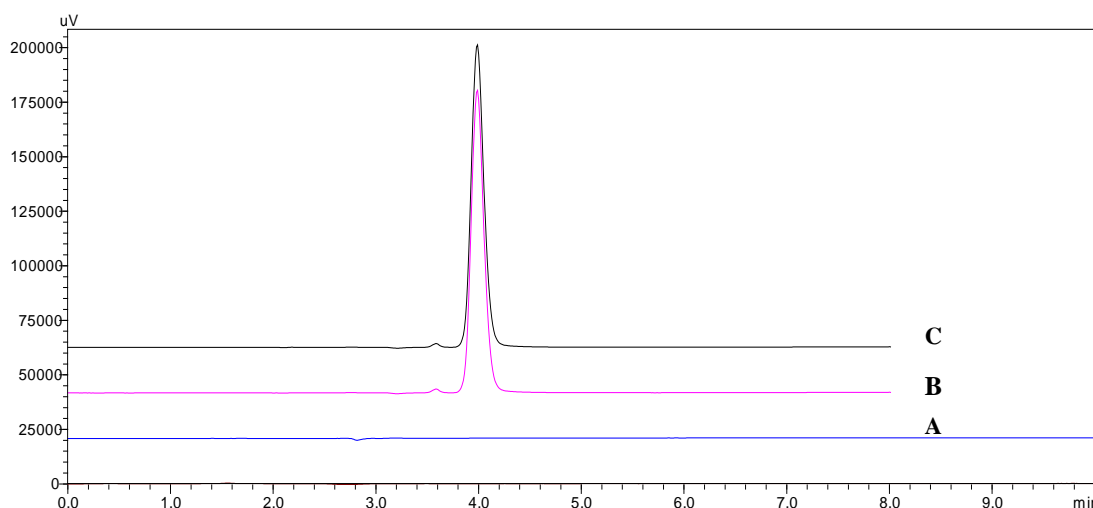


Fig. 5: A-mobile phase; B-standard chromatogram; C-sample chromatogram

Table 7: Assay Results (n=3)

Formulation	Labelled Amount (mg)	Amount found (mg) (Mean ± SD)	% Assay	% RSD
VAL Tablets (VALCIVIR® 500mg)	500	495.29 ± 0.073	98.98	0.074

CONCLUSION

It can be concluded that the proposed HPLC method is accurate, precise, sensitive and reproducible for the analysis of VAL in pharmaceutical dosage forms with shorter run time and is also economical.

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