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Research Article

Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Valacyclovir in Pharmaceutical Dosage Forms

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ABSTRACT

A rapid RP-HPLC method for determination of Valacyclovir in bulk and pharmaceutical dosage forms. Valacyclovir was found to be degraded under different set of conditions as followed according to ICH guidelines and the degradants so formed along with Valacyclovir are separated by using Hypersil BDS C18 150mm×4.6mm×5µm using mobile phase comprising of mobile phase-A (sodium dihydrogen phosphate monohydrate buffer P^H 3.51 with Orthophosphoric Acid) and mobile phase-B (acetonitrile: methanol, 60:40), with a flow rate of 1.5ml/min with a detection wavelength of 254nm with a injection volume of 10µl and the method was validated for specificity, linearity, accuracy, robustness and precision. The obtained results were indicating that the method is selective in analysis of both Valacyclovir in the presence of degradation products formed under various stress conditions.

Key Words: Valacyclovir, RP-HPLC, Validation, Stabilty.

INTRODUCTION

Valacyclovir is chemically (S)-2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate) the molecular formula $C_{13}H_{20}N_6O_4$ with a molecular weight of 324.336 g/mol. Valacyclovir is freely soluble in water and sparingly soluble in methanol DMF, DMSO and DMA, practically insoluble in ethanol, toluene and acetone. Valacyclovir[1] is an anti-viral, prodrug an esterified version of aciclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). Valacyclovir is converted by esterases to the active drug acyclovir, as well as the amino acid valine, via hepatic first-pass metabolism.

Acyclovir is selectively converted into a monophosphate form by viral thymidine kinase**,** subsequently; the monophosphate form is further phosphorylated into the active triphosphate form, aciclo-GTP by cellular kinases. Aciclo-GTP is a very potent inhibitor of viral DNA polymerase; it has approximately 100 times higher affinity towards viral than cellular polymerase. Its monophosphate form also incorporates into the viral DNA, resulting in the chain termination.

Literature review reveals that few analytical methods on Spectrophotometry[2,3,4], HPLC[5,6,7,8,9] were reported for Valacyclovir. The objective of the proposed method is to develop simple and accurate method for the estimation of Valacyclovir in pharmaceutical dosage forms by RP-HPLC.

MATERIAL AND METHODS

Chemicals and Reagents: Valacyclovir was obtained as a gift sample from Dr. Reddys Laboratory, Hyderabad, A.P. Acetonitrile (HPLC grade), Methanol (HPLC grade), Sodium dihydrogen phosphate monohydrate (AR grade) and Orthophosphoric acid (AR grade) were of reagent grade used.

Instrumentation: A HPLC (LC-2998, WATERS) with PDA detector, Hypersil BDS C_{18} 150mm×4.6mm×5µm column and auto sampler injector was used. The HPLC system was equipped with Empower2 software for data processing.

Chromatographic Condition: The mobile phase containing mobile phase-A i.e. sodium dihydrogen phosphate monohydrate buffer (P^H 3.51 with OPA) and mobile phase-B i.e. acetonitrile: methanol (60:40) was found to resolve Valacyclovir. Orthophosphoric acid was used for pH adjustment of buffer. The flow rate was set to 1.5ml/min. The drug shows good absorbance at 254 nm, which was selected as wavelength for further analysis.

Preparation of Dilute Phosphoric Acid solution: 5mL of Orthophosphoric Acid (85%) was transferred and diluted to 25mL with water.

Buffer preparation: 4.0g of Sodium Dihydrogen Phosphate anhydrous was transferred in to 1L of water and P^H was adjusted to 3.5 with dilute orthophosphoric acid.

Mobile Phase: Mobile Phase-A: P^H 3.5 buffer.

Mobile Phase-B: Mixture of Acetonitrile and Methanol in the ratio of 60:40v/v respectively.

Diluent: Mixture of Mobile Phase-A and Acetonitrile in the ratio of 90:10v/v respectively.

Preparation of Standard Solution: 60mg of Valacyclovir hydrochloride working standard (which was equivalent to 50mg of Valacyclovir) was accurately weighed and transferred into a 50mL volumetric flask. 40mL of diluent was added, sonicated to dissolve and made up to the volume with diluent.

Transferred 5mL of above solution into a 50mL volumetric flask and made up to the mark with diluent.

Preparation of Sample Solution: 20 tablets of Valacyclovir were weighed and powdered in a glass mortar. Transferred the powder equivalent to 500mg into

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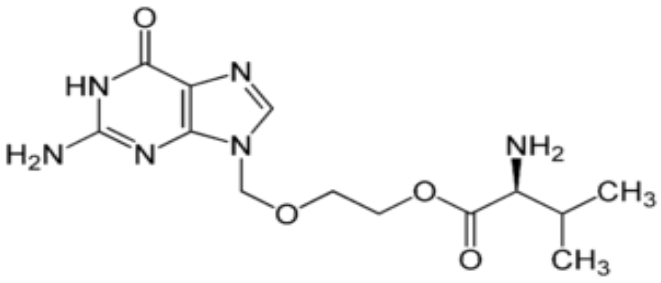

Fig.1: Structure of Valacyclovir

Table.1: Gradient Program

Time (min)	Flow Rate ml/min	%Mobile Phase-A	%Mobile Phase-B
0	1.5	97	3
6	1.5	95	5
8	1.5	65	35
12	1.5	40	60
13	1.5	97	3
18	1.5	97	3

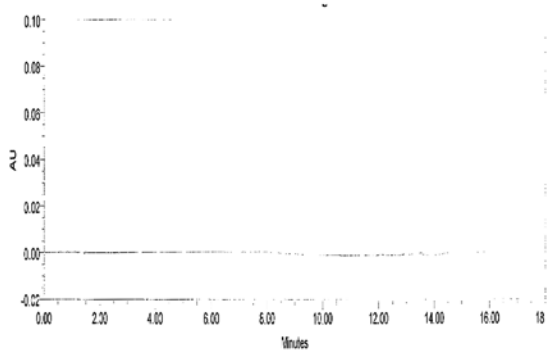

Fig.2: Blank Chromatogram

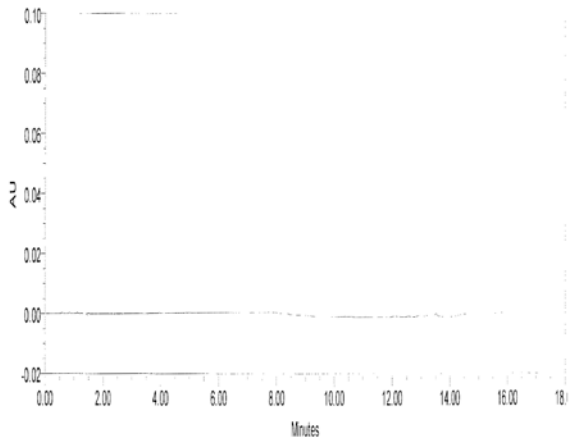

Fig.3: Placebo Chromatogram

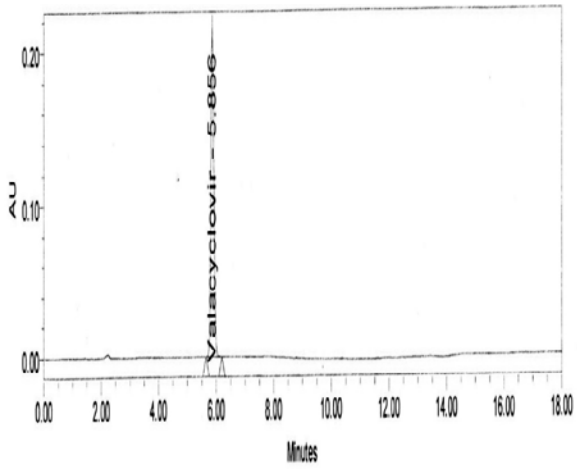

Fig.4: Standard Chromatogram

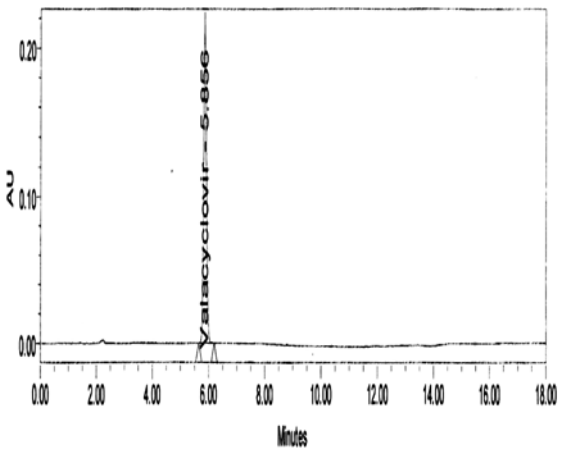

Fig.5: Sample Chromatogram

Table.2: System suitability data

S.No	Parameter	Observed value	Acceptance criteria
1	% RSD of peak area	0.5	NMT 2.0
2	Tailing factor	1.03	NMT 2.0
3	Theoretical Plates	>4000	NLT 3000

Table.3: Linearity Data

S.no	Conc. (ppm)	Peak Area
1	58.58	763484
2	87.87	1151484
3	117.16	1515649
4	146.45	1836687
5	175.74	2209237
6	slope	12211.37
7	Intercept	64624.6
8	Correlation cofficient	0.9996

500mL volumetric flask, 375mL of diluent was added and sonicated for 30min with intermediate shaking then made up to the volume with diluent. The above solution was centrifuged for 10min at 8000rpm. 5mL of the above solution was pipette out into 50mL volumetric flask and made up to the volume with diluent.

METHOD VALIDATION

1) System Suitability/System Precision: System Suitability was performed by injecting five replicate injections of standard solutions of Valacyclovir at 100% level (100µg/ml) and expressed as %RSD of peak area.

2) Linearity: [10] Standard solutions of various conc. of about 50%-150% level were injected into the chromatograph and calibration curve was constructed by plotting peak area against concentration in µg/ml.

3) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak, solutions of blank, Valacyclovir standard, sample, placebo and known impurities were prepared individually and run the chromatogram. The peak purity of analyte peak should be not less than 0.999.

4) Precision: [11] Precision was measured in terms of the repeatability of the application and measurement. Repeatability of standard application was carried out using six replicates of the sample injections.

5) Accuracy: [12] %Recovery studies were carried out at five different levels of 50%, 75%, 100%, 125% and 150% of standard solution (i.e. Valacyclovir API spiked to the placebo) in triplicate at each level.

6) Robustness[11,13]**:** The robustness of proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate (±0.3ml/min), column temperature ($\pm 5^0$c), filter variation (PVDF, Nylon), mobile phase variation (±10%) and Buffer P^H (±2) which may differ but the responses were still within the specified limits.

7) Ruggedness:The variability of the results obtained with the analysis of Valacyclovir sample six times by two different analysts, two different reagents, two different

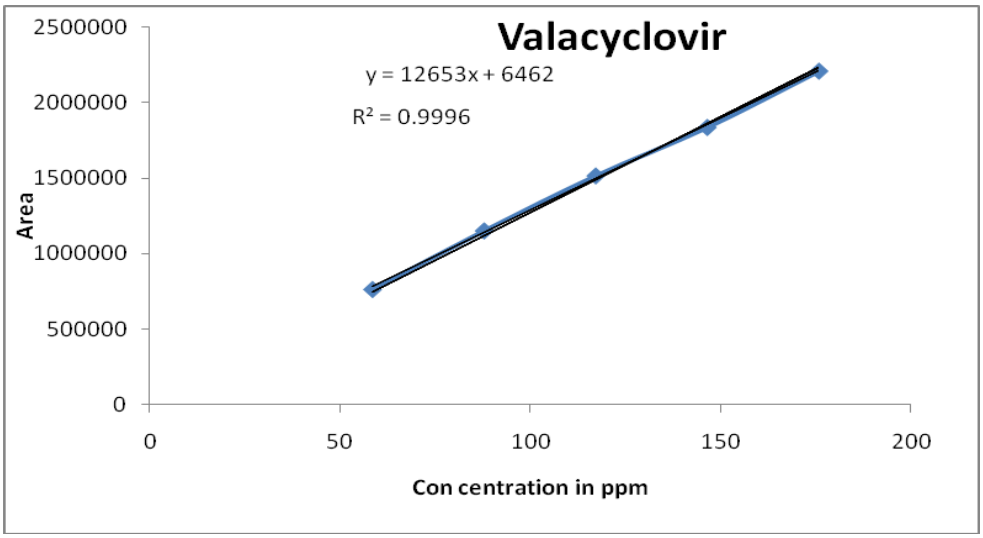

Fig.6: Calibration Curve for Valacyclovir

Table.4: Known impurities interference

S.No	Compound Name	Retention Time (min)
1	Valacyclovir	5.79
2	Guanine	1.48
3	Acyclovir	2.23
4	Alanine impurity	2.18
5	Isoleucine impurity	8.41
6	O-Acetyl acyclovir	7.76
7	N-Formyl Valacyclovir	8.99
8	CVCH1	11.75
9	CBZ-L-Valine (at 210nm)	12.00

Table.5: Precision Data

Sample Name	Peak Retention Time (Rt)	% Assay
Sample-1	5.88	98.33
Sample-2	5.88	99.99
Sample-3	5.87	99.48
Sample-4	5.86	98.62
Sample-5	5.86	100.27
Sample-6	5.86	99.98
%RSD	0.2	0.80

Table.6: Recovery Studies

Spike level (%)	Mean% recovery	% RSD
50	99.11	0.170
75	99.79	0.080
100	98.87	0.030
125	99.59	0.160
150	100.75	0.170

columns, two different instruments on two different days to assess the method ruggedness.

8) Bench top stability and Refrigerator stability: Standard and test solutions were prepared in duplicate by using Valacyclovir working standard and Valacyclovir HCl tablets. By kept the preparations on bench top and refrigerator these were injected at initial, after 1st day, 2nd day and 7th day and %assay measured.

forced degradation studies[14]

1) Acid Degradation: Blank, placebo and sample were refluxed in 1N HCl and neutralized by adding same amount of 1N NaOH. The final volume was made up with diluent. These preparations were heated for 2hrs at 60^0C and injected into the chromatographic system to observe degradation.

2) Base Degradation: Blank, placebo and sample were refluxed in 1N NaOH and neutralized by adding same amount of 1N HCl. The final volume was made up with diluent. These preparations were kept on bench top for 2min and injected into the chromatographic system to observe the net degradation.

3) Peroxide stress study: Blank, placebo and sample solutions were refluxed with 30%H_2O_2. They were heated for 1hr at 60^oC at injected into the chromatographic system to observe the net degradation.

4) Water stress study: Blank, placebo and sample were refluxed with water and kept for 1hr on bench top and then injected into the chromatographic system to observe the net degradation.

5) Sun light exposure study (visible light): Placebo and sample were exposed to sunlight for 55hrs and injected into the chromatographic system to observe the net degradation.

6) UV light exposure study: Placebo and sample were exposed to 200 wts /hr for 55hrs and injected into chromatographic system to observe the net degradation.

7) Heat stress study: Placebo and sample were exposed to 105^oC for 48hrs and injected into the chromatographic system to observe the net degradation.

8) Humidity stress study (about 90% RH at 25^0C): Placebo and sample were exposed to 90% humidity for 7 days and injected into the chromatographic system to observe the net degradation.

RESULTS AND DISCUSSION

To validate the RP-HPLC method, a series of tests were made using the most promising conditions and system suitability results were summarized in Table.2. A calibration curve was made and concentration examined within the detection range of 50-150µg/ml and correlation coefficient was found to be 0.9996, results were

Table.7: Robustness studies for Valacyclovir

S.NO	Conditions	%RSD
1	High Flow (1.8 ml/min)	1.1
2	Low Flow (1.2 ml/min)	0.2
3	Low Temperature (35ºc)	0.2
4	High Temperature (45ºc)	0.1
5	Low pH of buffer(3.3)	0.3
6	High pH of buffer(3.7)	0.4
7	Organic Phase (Acetonitrile) in mobile phase B (+2%)	0.4
8	Organic (Acetonitrile) in mobile phase B (-2%)	0.1

Table.8: Ruggedness Studies for Valacyclovir

S.No	System suitability	Observed value		Acceptance criteria
		Analyst-1	Analyst-2	
1	%RSD for Peak Area	0.5	0.5	NMT 2.0%
2	Tailing factor	1.03	1.23	NMT 2.0

Table.9: Bench Top Stability Data

Time in days	Similarity factor	% Assay of Sample		% Difference	
	Standard 2	Test-1	Test-2	Test-1	Test-2
Initial	1.00	99.99	99.48	NA	NA
1	0.98	100.51	102.19	0.52	2.71
2	0.98	102.36	101.18	2.37	1.7
7	0.99	101.17	101.29	1.18	1.81

Table.10: Refrigerator stability

Time in days	Similarity factor	% Assay of Sample		% Difference	
	Standard 2	Test-1	Test-2	Test-1	Test-2
Initial	1.00	99.99	99.48	NA	NA
1	0.98	100.87	102.18	0.88	2.7
2	0.98	102.36	101.09	2.37	1.61
7	0.99	98.72	99.17	1.27	0.31

Table.11: Stability Studies for Valacyclovir

S.NO	Stress condition	% Assay	% area of degradation	Peak purity angle	Peak purity threshold
1	1N HCL for 2hrs at 60°C	94.21	8.25	0.186	0.446
2	1N NaOH for 2min on BT	97.11	5.36	0.205	0.473
3	30% H_2O_2 for 1hr at 60^0C	95.29	7.18	2.339	26.978
4	BT	75.63	26.84	0.202	0.474
5	ırs	99.50	2.97	0.208	0.491
6	/hr	91.13	11.33	0.207	0.426
7		93.99	8.47	0.222	0.490
8	ɔr 7days	94.96	7.51	0.180	0.446

summarized in Table.3 and fig.2. There is no interference observed due to blank, placebo and impurities at retention time of analyte peak and the method is specific and chromatograms were shown in fig.3, 4, 5 and 6, results shown in table-4. The precision (expressed as the relative standard deviation) was determined for repeated analysis and the values are presented in Table.5. The recovery experiment values obtained were summarized in Table.6.

The reliability of the method was determined by conducting robustness and ruggedness studies and results were summarized in Table.7, 8 and 9 and solution stability of analyte was studied by storing the drug in refrigerator and on bench top and results was given in table.10 and 11.

The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated and given in table.12.

CONCLUSION

A method was developed for the determination of Valacyclovir in pharmaceutical dosage forms which is rapid, accurate & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

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