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Development and validation of bioanalytical method for the determination of Valcyclovir HCl in human plasma by liquid chromatoghraphy

Mahesh Mukund Deshpande*, Veena Sanjay Kasture, Mahalaxmi Mohan, Sanjay Chaudhari

Jawaharlal Nehru Technological University, Hyderabad, Amrutvahini College of Pharmacy, Sangamner, Sp Pune University, Pune

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Abstract

Valacyclovir hydrochloride (2-[(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxyl]ethyl ester-l-valine monohydrochloride), is an antiviral prescription medicine approved by the U.S. Food and Drug Administration (FDA) to prevent genital herpes outbreaks in adults infected with HIV.A simple method is described for the quantitation of Valcyclovir HCl in plasma by liquid chromatography. Chromatographic separation was achieved on a reversed phase Hypersil ODS C18 (150mm * 4.6mm, 5.0 µm) column, using isocratic elution (acetonitrile-water (85:15) at a flow rate of 0.2–1.2 mL min^{-1}. Valacyclovir hydrochloride were measured using UV detection at 265 nm. The total chromatographic run-time was 10 min with Valacyclovir hydrochloride eluting at 4.19 min. Limit of quantification was 50 ng mL^{-1}. The linearity range of the method was 50-2000 ng mL^{-1} ($r2$ = 0.9987). Mean recoveries from plasma were 105.13%. Intra-batch and inter-batch precision was 0.857 and 0.842, respectively. The Freeze and Thaw Stability , Short-Term Temperature Stability , Long-Term Stability, Stock Solution Stability evaluation indicated no evidence of degradation of Valacyclovir hydrochloride. The validated method is simple, selective and rapid and can be used for pharmacokinetic study.

Keywords:

Valacyclovir hydrochloride; Human plasma; Stability

1. Introduction

Valacyclovir hydrochloride (2-[(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxyl] ethyl ester-l-valine monohydrochloride), is an antiviral prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment and prevention uses of certain types of herpes simplex virus (HSV) like initial or recurrent episodes of genital herpes in healthy adults, genital herpes outbreaks in adults infected with HIV, reduce the risk of transmitting genital herpes to other people, treat cold sores (also known as herpes labialis or labial herpes) in adults and children. Literature survey revealed that few spectrophotometric methods, HPLC methods, and LC-MS methods for biological fluid are reported in the literature for the determination of valcyclovir HCl in Bulk, pharmaceutical formulations and serum samples [1-9]. We describe here the development of new HPLC method for the determination of valcyclovir HCL in Plasma and its stability data, which can be applied for pharmacokinetic study of valcyclovir HCL.

* **Corresponding Author**
E-mail: maheshdeshpande83@gmail.com
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2. Experimental

2.1. Chemicals

HPLC grades Acetonitrile, water were purchased from Merck specialties pvt. Ltd. Plasma was obtained as a gift sample from Red Cross Blood Bank. Water purified on Millipore was used. All other chemicals were of analytical grade.

2.2. Instrumentation

A Shimadzu HPLC system with LC solutions software equipped with UV detector, Hypersil ODS C18 (150mm * 4.6mm, 5.0μm) column, Digital Ultra Sonic Cleaner (heater), Shimadzu Electronic Balance Type BL-220H, REMI Cooling Centrifuge C24 were used.

2.2.1. Chromatographic conditions

The mobile phase ratio was optimized in isocratic mode for the analysis of valcyclovir HCL. Different ratios were studied, such as 50:50, 60:40, 80:20, 85:15 and 90:10 of acetonitrile: water for valcyclovir HCL. The final mobile phase consisted of acetonitrile-water (85:15). The mobile phase was always clarified by filtration through a nylon filter paper, with pore size equal to 0.45μm, and degassed through a sonicator, then pumped at flow rate of 0.5 ml/min, in isocratic mode on Hypersil ODS C18 column (150 mm ×4.6 mm, 5μm). The peak response was monitored at a wave length of 265nm. The sample (20 μL) was injected into HPLC system and the data was acquired using LC solutions software

2.3. Preparation of standard solutions

A stock solution of valcyclovir HCL was prepared in a volumetric flask by dissolving accurately weighed amount of valcyclovir HCL in acetonitrile,to give 0.1 mg mL^{-1} concentration. Working standard solution of valcyclovir HCL(50-2000 μg mL^{-1}) were prepared by appropriately diluting the respective stock solution with acetonitrile. All stock and working standard solutions were stored at 4°C until used for analysis. Calibration curve was constructed for the standard solutions containing valcyclovir HCL in the range of 50-2000 ng mL^{-1} at 50, 100, 500, 1000, 1500, 2000 ng mL^{-1} after HPLC/UV analysis.

2.4. Plasma sample processing

A volume of 0.5 mL of drug-free plasma was transferred to a centrifuge tube and spiked with 0.5ml of appropriate working standard solution of valcyclovir HCL to achieve standard solutions containing 0.1-4 μg mL^{-1} of valcyclovir HCL in plasma. Blank plasma samples were spiked with mobile phase. A single–step protein precipitation method was followed for extraction of valcyclovir HCL from human plasma. Each of the above plasma samples were mixed for 1 min and then subjected to protein precipitation by adding 1 mL of acetonitrile and mixed thoroughly for 1 min. Samples were then centrifuged at 4000 rpm, 4°C for 20 min. A clean and clear supernatant was transferred to a sample loading vial. This was filtered using 0.45 μ nylon membrane filter paper operated via vacuum filtration and injected into the HPLC system.

2.5. Bioanalytical method Validation

The method was validated according to FDA guidelines for validation of bioanalytical methods in order to show acceptable nature of analytical proposed analytical method was validated according to standard guidelines with respect to the following parameters.

2.5.1 Selectivity

The selectivity of the method was evaluated by processing drug-free plasma samples and the plasma calibration standards. Chromatograms were compared for any interference from the matrix or any of the assay reagents.

2.5.2. Accuracy and precision

Intra-batch accuracy and precision were determined by analysis of six replicates of the low, medium and high concentration QC samples; while inter-batch accuracy and precision were determined by the analysis of these QC samples on three separate occasions. The overall precision of the method was expressed as relative standard deviation (R.S.D.) and the accuracy of the method was expressed as mean.

2.5.3. Recovery

Recovery was performed by comparing the concentrations of three QC samples (n=6 replicates) at low, medium and high with the unextracted reference standards containing the same amount of the analyte. With regard to the preparation of the unextrcted reference standards, plasma was precipitated using acetonitrile, vortexed and centrifuged. Supernatent was drawn off, to which standard and internal standard were added (n=6), dried and reconstituted with mobile phase for analysis.

2.5.4. Linearity

A calibration curve was prepared from a six calibration samples covering the range (50 – 2000 ng mL^{-1}); including the lower limit of quantitation (LOQ).the acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value, except the LOQ, which was set at 20 %.

2.5.5. Sensitivity

The lowest standard on the calibration curve was identified as the lower limit of quantitation (LOQ) as the analyte peak was identifiable, discrete and reproducible with a precision of less than or equal to 20% and accuracy of 80-120%.

2.5.6. Stability

The freeze-thaw stability of the samples was obtained over three freeze-thaw cycles, by thawing the QCs unassisted at room temperature and then refreezing for 12-24 h followed by analysis. Short term stability was evaluated by keeping the QCs at room temperature for 24 h and then reanalyzing. Long-term stability was analyzed at three replicates of low and high concentrations on first and sixth day.

2.5.7. Stock Solution Stability

The working solution of valcyclovir HCL were determined immediately after preparation (time 0) and at 6 h at room temperature and 4^0 C and concentration were compared.

3. Results and Discussion

3.1. Chromatography Method

The chromatographic conditions were optimized to provide acceptable resolution of the analytes present in the plasma matrix. Mobile phase selection was based on the peak parameters, run time and ease of preparation. The isocratic condition of acetonitrile-water (85:15). Provided good resolution of valcyclovir HCL (RT~ 7 min) Fig.2 and 3 shows representative chromatograms of blank plasma, calibration plasma with valcyclovir HCL. No

interference peaks were observed in drug free plasma. The limit of quantitation of valcyclovir HCL was 50 ng mL^{-1}. The precession and accuracy for the LOQ were 0.857, 0.842 %, respectively. The calibration curve of valcyclovir HCL was linear over the concentrations range from 50-2000 ng mL^{-1} with mean r^2 = 0.9987, n=3 (Table 1).

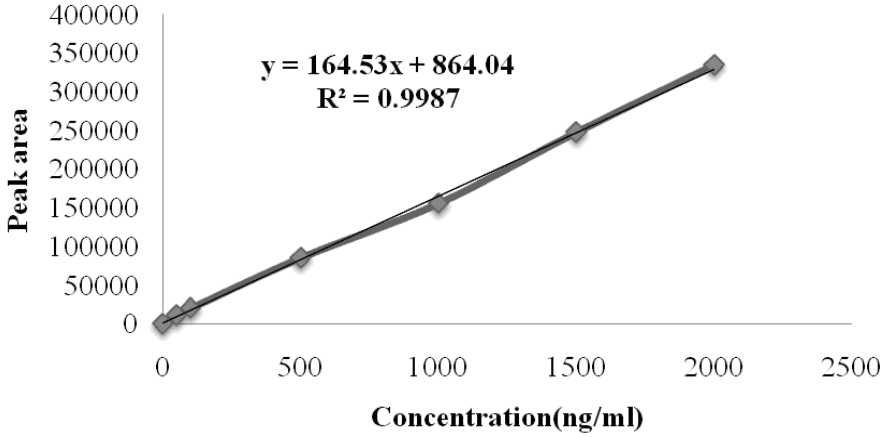

Fig- 2: Calibration curve for Valcyclovir HCL in human plasma

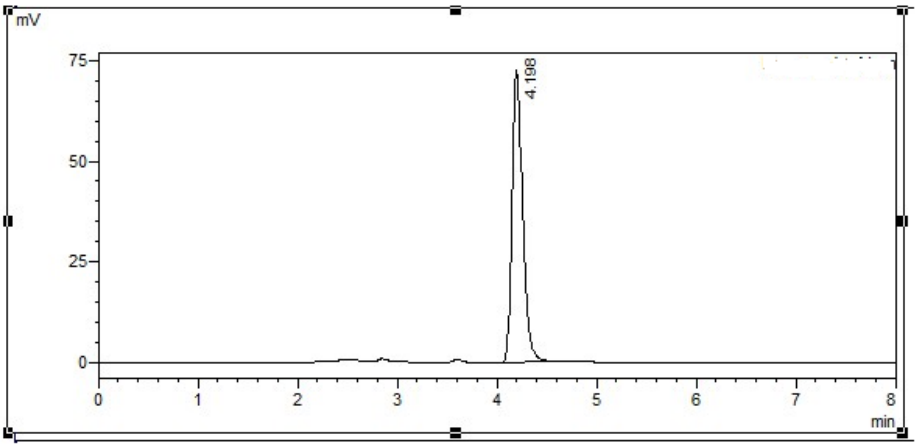

Fig.3: Chromatogram of Valcyclovir HCL in human Plasma

Table 1: Linearity and System Suitability Parameter

Parameter	Valcyclovir HCL
Linearity range(ng/ml)	50-2000 ng mL^{-1}
Correlation co-efficient	0.9987
Slope	164.53
Intercept	864.04
Theoretical plates	2791.434
Tailing factor	1.41

3.2. Recovery

The mean relative recovery of valcyclovir HCL from the plasma was 98.81% with the coefficient of variation 1.94 % at three different concentrations (25 ng mL^{-1}, 50 ng mL^{-1}, and 75 ng mL^{-1}) (Table 2)

Table 2: Recovery of valcyclovir HCL (n=3)

Concentration (ng mL^{-1})	%Recovered	Avg. (%)
25	133.6 136 132.2	133.9%
50	100 97.6 99.6	99.06%
75	90.9 97.5 92.4	93.60%
Mean	108.85%	

3.3. Accuracy and precession

Intra- and inter-batch precision and accuracy were evaluated by assaying the three QC samples. Intra-and inter-batch precision (% CV) was 0.857 and 0.842 respectively. Accuracy of the proposed method was determined on the basis of percent recovery at three concentration levels 500, 1000 and 1500 ng mL^{-1}. The average percent recovery for valcyclovir HCL was found to be 105.13 % (Table 3 and 4).

3.4. Stability

Stability results are presented in table 4.short term storage stability at room temperature and freeze-thaw cycles, long term stability for low and high QC samples indicated that valcyclovir HCL was stable in plasma under experimental condition. The stock solution stability of valcyclovir HCL stored at 4 ºC and room temperature over 24 h showed no evidence of degradation. Valcyclovir HCL was recovered at storage conditions indicating their stability under laboratory working conditions. (Table 5)

Table3: Precision for determination of valcyclovir HCL from Plasma

Concentration added (ng mL^{-1})	Concentration found (ng mL^{-1}) (mean ± SD, n = 6)		% R.S.D.
5	5.6	± 1.8	1.94
14.7	14.7	± 0.6	0.85
85.7	85.9	± 3.4	0.87
950	951.3	± 15.7	1.25

Table 4: Accuracy for determination of valcyclovir HCL in human plasma at low, medium and high concentrations (n=3)

Concentration (ng mL^{-1})	Recovery, %	Average, %
500	109.3 109.3 109.9	109.46
1000	100 99.7 99.89	99.86
1500	105.8 106.05 106.4	106.08
Mean	105.13	

Table 5: Stabilty of valcyclovir HCL

Parameter	Concentration (ng L^{-1})	Concentration found (ng L^{-1}) (mean ± SD	CV
Short term Stock solution stability (6Hr.)	LQC	14.5 ± 1.4	8.09
	MQC	84.7 ± 2.5	6.34
	HQC	952.4 ± 3.6	0.82
Long term Stock solutionstability (6 days)	LQC	13.8± 1.3	4.24
	MQC	81.6± 1.5	5.3
	HQC	946.3± 5.2	3.83
Batch top stability	LQC	14.8± 2.8	7.19
	MQC	82.0± 3.5	1.15
	HQC	951.8± 5.6	1.39
Freeze –thaw stability	LQC	14.9± 2.3	9.05
	MQC	85.2± 3.4	6.63
	HQC	959.6± 2.1	3.06
Long term stability	LQC	14.6± 3.1	2.75
	MQC	85.5± 5.2	3.79
	HQC	952.3± 2.5	5.37

(LQC, MQC, HQC-low, medium, high QC samples)

Conclusions

A simple, selective and rapid HPLC method with UV detection was developed for determination of valcyclovir HCl in plasma samples. Validation was performed as per guidelines and all parameters met the criteria acceptable for routine analysis. The method can be used for routine analysis of valcyclovir HCL for its pharmacokinetics study.

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References

1. United States Pharmacopeia, National Formulary-29, Rockville, USA: United States Pharmacopeial convention; 2011.
2. Maryadele J, The Merck Index, In: O'Neil, editor. 13th ed. Whitehouse Station, NJ, USA: The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 2001, 9966.
3. Martindale, the Extra Pharmacopoeia, Published by direction of the Council of Royal Pharmaceutical Society of Great Britain, 34th ed. Vol. 11. London Royal Pharmaceutical Society, 1996, 2005, 653,656.
4. Kasiari M, Gikas E, Kazanis M, Panderi I, Journal of Chromatography B, 864(1–2), 2008, 78-86.
5. Heon-Woo L, Ji-Hyung Seo, Kyung-Tae Lee, Journal of Chromatography B, 852(1–2), 2007, 382-388.

6. Sasanya JJ, Abd-Alla AM, Parker AG, Cannavan A, Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 878(26), 2010, 2384-2390.
7. International Conference on Harmonisation, Validation of analytical procedures: Text and methodology, In ICH Harmonized Tripartite Guidelines Q2 (R1), November 2005
8. Surendra Bansal and Anthony DeStefano , The AAPS Journal 2007; 9 (1)
9. Henning Blumea,c, Erich Brendelb Margarete Brudny-Klöppelb , Sylvia Grebec, , Berthold Lauseckerd ,Gabriele Rohdeb, Christoph Siethoff , European Journal of Pharmaceutical Sciences 42 (2011) 300–305.