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HPLC method development, validation, and determination of valganciclovir

R.Radha[1],K.Kavi Priya[1],M.Kishore Babu[1],V.S.Chandrasekaran[1],B.Swapna[1]

V .Anitha Kumari [2]

[1]Department of Pharmaceutical Analysis,Krishna Teja Pharmacy college,Tirupati - 517506

[2] Department of Pharmaceutical Analysis ,Sir C.R.Reddy college of Pharmaceutical Sciences ,Eluru – 534007

ABSTRACT

Valganciclovir is an antiviral medication used to treat cytomegalovirus (CMV) retinitis in patients diagnosed with acquired immunodeficiency syndrome (AIDS). An isocratic high performance liquid chromatography method has been developed for routine quality control of Valganciclovir. Separation was carried out by C-18, 250 x 4.6 mm, 5 um (X-Bridge, Waters) column using mobile phase Ammonium Acetate Buffer pH 3 (adjusted with glacial acetic acid) and methanol (55:45 v/v) at a flow rate of 1 ml/min. The detection was carried at 254 nm at ambient temperature. Excellent linear relationship between peak area and Valganciclovir concentration in the range of 5-30 µg/ml has been observed (r^2, 0.998). Developed method has been found to be sensitive (limits of detection and quantification were 0.52µg/ml and1.58 µg/ ml, respectively), The % Assay was found to be 101.15 % ± 0.653, specific and robust (% RSD were less than 2 %, for system suitability parameters).

Keywords:valganciclovir, Antiviral, HPLC, method development, Validation

INTRODUCTION:

Valganciclovir hydrochloride is an antiviral medication used to treat cytomegalovirus infections. As the L-valyl ester of ganciclovir, it is actually a prodrug for ganciclovir. After oral administration, it is rapidly converted to ganciclovir by intestinal and hepatic esterases. Chemically, Valganciclovir[2-[(2-amino-6-oxo-3H-purin-9-yl)methoxy]-3-hydroxypropyl] (2S)-2-amino-3-methylbutanoate.(Fig.1)

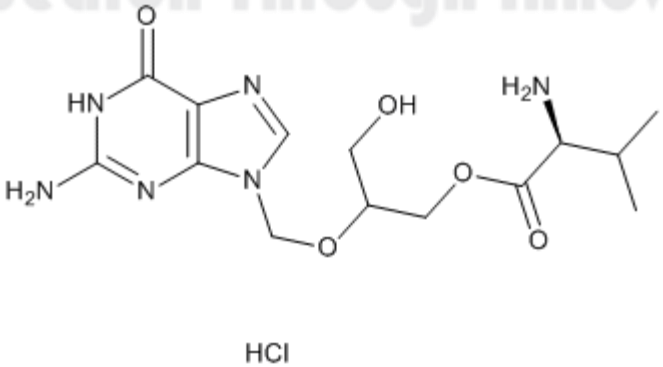

Fig.1. Chemical structure of Valganciclovir

Few methods are reported for estimation Valganciclovir of which include HPTLC[1], stability indicating HPLC[2], LC-MS/MSand UV spectroscopic methods[3]. No methods are reported for development and validation of HPLC method for estimation of valganciclovir. This work describes the development, validation and application of a reliable, simple, accurate and robust HPLC method.

MATERIALS AND METHODS

Chemicals and Reagents:

Chemicals and reagents like Chloroform, Methanol, Ammonium Acetate, glacial acetic acid a (All HPLC grade) was purchased from LOBA CHEMICAL PVT. LTD, Mumbai.

Instrumentation and chromatographic conditions

Analysis was carried out on Jasco HPLC system with PDA detector using Borwin software. Separation was carried out on C-18, 250 x 4.6 mm, 5 um (X-Bridge, Waters) column using mobile phase Ammonium Acetate Buffer pH 3 (adjusted with glacial acetic acid) and methanol (55:45 v/v) at a flow rate of 1 ml/min. The detection was carried at 254 nm at ambient temperature.

Preparation of Standard stock solution:

Standard stock solution of drugs was prepared by dissolving 10 mg of drug in methanol to get concentration of 1000 µg/ml (A). From this working standard solution was prepared containing 100 µg/ml of Valganciclovir(B). From this further dilution was made in methanol to get 10 µg/ml as final solution of Valganciclovir.

Selection of Detection Wavelength:

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 254 nm. Representative UV spectrum of Valganciclovir is shown in Fig.2.

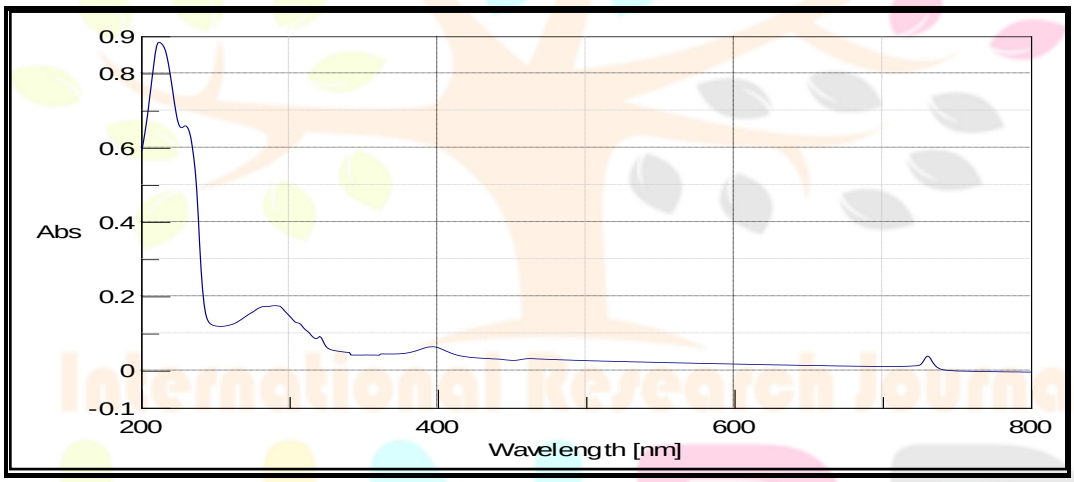

Fig. 2: UV Spectrum of Valganciclovir(10 µg/ml)

Validation of the method [14]

The analytical method was validated with respect to parameters such as linearity and range, precision, accuracy, robustness, specificity, LOD and LOQ

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 996 [Purity front 996.48 and Purity tail 996.72], indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µg/ml) of Valganciclovir, solution was prepared containing 100 µg/ml of Valganciclovir with methanol. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml. The equation of calibration curve was found to be y = 44685x + 110741. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig.3. The results obtained are shown in Table 1.

Table 1: Linearity study of Valganciclovir

Replica	Concentrations of Valganciclovir					
	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml
	Peak Area					
1	368998.9	491014.3	826249.3	939266.3	1205953	1486319
2	367712.9	491752.7	828871.4	937615	1210782	1499969
3	379901.5	506678.9	848199.4	940854.8	1219348	1479597
4	383917.8	511752.7	867857.5	954219.6	1240568	1500149
5	369913.7	497237	836124.7	938617.7	1205380	1485219
6	367736.5	507237	837793	899934.8	1233571	1471654
Mean	373030.2	500945.4	840849.2	935084.7	1219267	1487151
Std. Dev.	7042.94	8787.95	15308.55	18282.91	14835.54	11268.48
%RSD	1.888	1.754	1.821	1.955	1.217	0.758

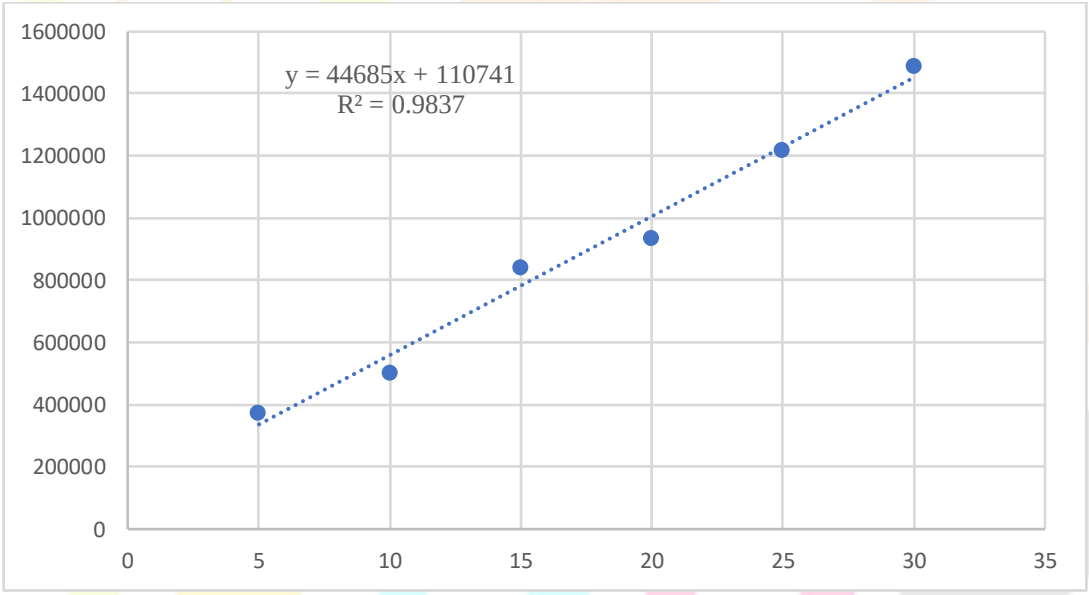

Fig. 3: Calibration curve of Valganciclovir (5-30 µg/ml)

Precision:

The precision of the method was demonstrated by intraday and interday variation studies. In the Intraday studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the interday variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intraday and interday variations are shown in Table 2a and 2b, respectively.

Table 2a: Intra-day precision study of Valganciclovir

Replicates	Conc. (µg/ml)		
	15	20	25
1	792249.27	989866.3	1217952.50
2	788871.4	1021915	1218009.65
3	788199.39	997818.8	1217747.65

Mean	789773.4	1003200	1217903
SD	0.323	1.867	0.012
% RSD	0.319	1.869	1.244

Table 2b: Inter-day precision of Valganciclovir

e	**Conc. (µg/ml)**		
	15	**20**	**25**
1	788199.38	990854.8	1219347.65
2	786857.47	1014219.6	1240567.8
3	791124.66	1018617.7	1215380
Mean	788727.2	1007897	1225098
SD	0.325	1.669	1.212
%RSD	0.321	1.663	1.215

Assay:

Twenty tablets [V-GAVIR (450 mg)] were weighed and the contents were finely powdered. Powder equivalent to 10 mg of Valganciclovir was accurately weighed and transferred into a 10 ml volumetric flask and volume was made up with methanol as mentioned under section preparation of stock solution (1000 µg/ml). The volumetric flask was sonicated for 10 min to enable complete dissolution of Valganciclovir and the solution was filtered through 0.45 µm whatmann filter paper. From the filtrate further dilution was made with methanol to get 100 µg/ml solution. Finally, this solution was further diluted with mobile phase to yield a concentration of 10 µg/ml and then it is injected. The procedure was repeated for six times. The results obtained for assay of Valganciclovir are shown in Table 6.8.

Table 3: Assay of marketed formulation

	VALGANCICLOVIR		
Sr. No.	**Peak area**	**Amount Recovered (µg/ml)**	**% Recovery**
1	559821.7	10.04	100.50
2	559794.1	10.04	100.49
3	566360	10.19	101.96
4	560647.7	10.07	100.68
5	565077.4	10.17	101.67
6	564608.5	10.16	101.57
Mean	562718.2	10.12	101.15
SD	2953.93	0.066	0.661
%RSD	0.52	0.65	0.653

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablet solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml. Percentage recovery was determined from linearity equation. (Table 4)

Table 4: Recovery studies of Valganciclovir

Level % of Accuracy	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	% RSD
50 %	10	5	791086.132	15.23	101.66	1.000
			785960.941	15.11		
			799466.487	15.41		
100 %	10	10	1020793.01	20.36	101.87	0.036
			1021457.84	20.38		
			1021158	20.37		
150 %	10	15	1219952.51	24.82	100.40	1.021
			1234493.83	25.15		
			1242567.8	25.33		

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined from linearitydata using the formula, LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = Standard deviationof y-intercept and S = Slope of the calibration curve. LOD and LOQ were found to be 0.52µg/ml and 1.58 µg/ ml, respectively

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate of mobile phase was altered and the effects on the area were noted. The method was found to be robust. The results obtained are shown in Table 5.

Table 5: Robustness study

% RSD Found for Robustness Study(peak area)								
MP COMPOSITION			**DETECTION WAVELENGTH (± 1 nm)**			**FLOW RATE (± 0.05 ml/min)**		
53:47	**55:45**	**57:43**	**253**	**254**	**255**	**0.95**	**1**	**1.05**
1.25	1.28	1.61	1.28	1.28	1.28	1.78	1.28	1.29

CONCLUSION:

The proposed HPLC method was developed and validated as per the ICH guidelines. Validation results proved that the developed method performs well with selectivity, precision; accuracy, stability and linearity.

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