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Development and Validation of Stability Indicating RP-HPLC Method for Quantitative Determination of Valganciclovir in Pure and Pharmaceutical Formulations

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ABSTRACT

The development and validation of a new stability indicating RP-HPLC method of valganciclovir in pure and dosage forms by RP-HPLC was described. Valganciclovir was separated isocratically on an µBondapak® C18 (250 X 4.6 mm), 5µm column with a mobile phase consisting of an isocratic mobile phase containing 0.01M sodium dihydrogen phosphate buffer (pH 5.0) and acetonitrile in the ratio of 600:400 v/v was carried out with the flow rate of 1.2 mL/min at ambient column temperature. The effluent was monitored at 254 nm. All the analysis was carried out at 35°C respectively. The developed RP-HPLC method extensively validated as per ICH standards and the results of the above investigations are included in this part respectively.

INTRODUCTION

Valganciclovir[1-4][Fig.1] is the hydrochloride salt of the L-valyl ester of ganciclovir. Ganciclovir is a synthetic nucleoside analogue of guanine is used in the treatment of Cytomegalovirus (CMV) retinitis in patients with acquired immunodeficiency syndrome (AIDS) and also prevention of CMV disease in kidney, heart, or kidney-pancreas transplant patients at high risk.

All previously published methods[5-18] reported in the literature suffered from drawbacks of long run times and complex mobile phases. In the view of the importance of stability testing, the author developed and validated a stability-indicating RP-HPLC assay method for valganciclovir in pure and pharmaceutical dosage form as per ICH guidelines. The present section of this chapter describes the the author's investigations and the experimental work carried out in the development of a validated stability indicating analytical RP-HPLC method for the assay of valganciclovir in pure and dosage forms.

MATERIALS AND METHODS

Experimental

i. Instrumentation: The HPLC analysis of valganciclovir was carried out on a Shimadzu 2010C integrated high performance liquid chromatographic system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The µBondapak® C18 (250 X 4.6 mm), 5 µm was used as a stationary phase, maintained at 25°C. The injection volume of sample was 20 µL. The photodiode array UV-detector was set to a wavelength of 254 nm for the detection and chromatographic run time was 10 minutes. The entire HPLC system was equilibrated before making each injection. Shimadzu balance (BL-220H) was used for all weighing.

ii. Chemicals and Solvents: Valganciclovir standard (99.9 % pure) was obtained as gifted sample from Laboratory, Mumbai. Tablets of valganciclovir [VALCYTE-450mg] were purchased from local pharmacy. Acetonitrile (HPLC grade), Orthophosphoric acid (GR Grade), Sodium dihydrogen phosphate monohydrate (GR Grade) and Tri ethylamine (GR Grade) were purchased from Qualigens Ltd., Mumbai. The purified water prepared by using a Milli- Q system was used for the preparation of buffer and other aqueous solutions.

iii. Mobile Phase Preparation: Prepare a filtered and degassed mixture of buffer (pH 5.0) and acetonitrile in the ratio of 600:400 v/v was used as mobile phase in current assay respectively.

Buffer Preparation: Accurately weigh and transfer about 2.72 gms of Sodium dihydrogen phosphate (monohydrate) and 2.0 mL of triethylamine in 1000 mL of purified water and mix. Adjust pH to 5.0 (±0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45 µm membrane filter.

iv. Diluent Preparation: In the present study mobile phase is used as diluent.

v. Preparation of Standard Solution: Accurately weighed about 100.0 mg of valganciclovir and transferred into a 100 mL volumetric flask then, added 60 mL of diluent and sonicated to dissolved. Cooled the solution to room temperature and diluted to mark with methanol [stock solution]. Transferred aliquots of the above solution [stock] into series different 100 mLvolumetric flasks and diluted to volume with the diluent respectively to give concentrations range 5.0 – 30.0 $\mu g.mL^{-1}$. 20 µL of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

vi. Analysis of Marketed Sample (Dosage Forms): Ten tablets of valganciclovir (VALCYTE-450 mg) were purchased from local pharmacy, weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of valganciclovir was transferred to a 100 mL volumetric flask followed by the addition of 70 mL of methanol. The solution was sonicated at controlled temperature for 30 min and diluted to volume with methanol and mixed thoroughly. Filter the solution through 0.45 µm membrane filter. Prepare different working sample solutions in the concentration range 5.0 – 30.0 $\mu g.mL^{-1}$ by diluting the above solution into a series of 100 mL volumetric flasks and diluted to volume with diluent. 20 µLof these solutions were injected in triplicate into HPLC system and preceded as said for the standard respectively.

RESULTS & DISCUSSION

i. Method Development: Critical parameters, such as wavelength of detection, composition of mobile phase, optimum pH, and concentrations of the standard solutions were studied in detail to develop an effective method for quantification of valganciclovir. To obtain the absorbance

maxima, solutions containing valganciclovir were prepared in various solvents and run through an ultraviolet spectrophotometer in the wavelength range of 190–400 nm. A wavelength of 254 nm was established as the λ_{max}, and was obtained when the solution was scanned in acetonitrile, methanol, and mobile phase. The chromatographic parameters were evaluated using a variety of columns, including C18 and C8, and a µBondapak C18 was selected as the most appropriate (note that with the C8 column the peak is asymmetrical and has a small peak shoulder). Mobile phase was developed using buffer in combination with acetonitrile or methanol. It was found that acetonitrile gave a sharp symmetric peak with a shorter run time than methanol. Similarly, the buffer and acetonitrile ratio was optimized to identify the optimal mobile phase, which had a short run time of 10 minutes without any interference, and was used in the subsequent analysis. The validative chromatogram and the system suitability results of the present developed RP-HPLC method for valganciclovir were presented in Fig.2 and Table.1 respectively.

Chromatographic Conditions: The compound was separated isocratically on an µBondapak® C18 (250 X 4.6 mm), 5 µm column with a mobile phase consisting of an isocratic mobile phase containing 0.01M sodium dihydrogen phosphate buffer (pH 5.0) and acetonitrile in the ratio of 600:400 v/v was carried out with the flow rate of 1.2 mL/min at ambient column temperature. Before the analysis, the mobile phase was degassed and filtered through a 0.45 µm membrane filter. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore) and degassed. The effluent was monitored at 254 nm. All the analysis was carried out at 35°C respectively.

ii. Forced Degradation:

a) Control Sample: Weighed and finely powdered not fewer than 20 tablets. Accurately weigh and transfer powder equivalent to 50 mg of valganciclovir into a 100 mL volumetric flask, containing 70 ml of diluent, sonicated for 30 minutes with intermittent shaking at controlled temperature and finally diluted to the mark with methanol and mixed. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 100 ml volumetric flask and diluted to volume with the same diluent.

b) Acid Degradation Sample: Accurately weighed and transferred powder equivalent to 50 mg of valganciclovir into a 100 mL volumetric flask, containing 70 mL of methanol, and sonicated

for 30 minutes with intermittent shaking at controlled temperature. Then added 10 mL of 5N acid to the same flask and refluxed for 30 min at 60°C, then cooled to room temperature, and neutralized with 5N NaOH and finally diluted to volume with methanol and mixed. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask and diluted to volume with diluent.

c) Base Degradation Sample: Accurately weighed and transferred powder equivalent to 50 mg of valganciclovir into a 100 mL volumetric flask, containing 70 mL of methanol, and sonicated for 30 minutes with intermittent shaking at controlled temperature. Then added 10 mL of 5N Base (NaOH), refluxed for 30 min at 60°C, cooled to room temperature, neutralized with 5N Acid (HCl) and diluted to volume with methanol and mixed. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask and diluted to volume with diluent.

e) Thermal Degradation Sample: Powders collected from 20 tablets were exposed to heat at 105°C for about 5 days. Accurately weighed and transferred equivalent to 50 mg of valganciclovir into a 100 mL volumetric flask, added about 70 mL of methanol, and sonicated for 30 minutes with intermittent shaking at controlled temperature and diluted to volume with methanol and mix. Filtered the solution through 0.45 µm membrane filter. Transferred 5.0 mL of the above solution into a 100 mL volumetric flask and diluted to volume with diluents.

Similarly UV-light exposure stress samples are prepared and checked for their purity by proposed method. From the results of degradation studies it was observed that no degradation was observed and the results, chromatograms of various degradation studies of valganciclovir revealed the suitability of the developed RP-HPLC method to study stability of dexamethasone under various forced degradation conditions viz. acid, base and oxidative degradation.

A. Method Validation: The developed RP-HPLC method is extensively validated for assay of valganciclovir in pure and formulation in accordance to ICH guidelines by using the following parameters.

i. System Suitability: The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were calculated. It was observed that all the values

are within the limits (Table.1). These values revealed the feasibility of the developed method for routine pharmaceutical analysis for valganciclovir.

ii. Specificity/ Selectivity: The selectivity of the present RP-HPLC method was evaluated by injecting the placebo, blank and pure drug solution into the chromatographic system under the above said optimized chromatographic conditions and their respective chromatograms were recorded. Chromatogram of blank solution showed no peaks at the retention time of valganciclovir peak indicating that the diluent solution used in sample preparation do not interfere in the assay of valganciclovir. Similarly chromatogram of placebo solution showed no peaks at the retention time of valganciclovir peak revealing that the placebo used in sample preparation do not interfere in assay of valganciclovir in pure and formulations.

iii. Linearity: The calibration curve for valganciclovir standard solutions in a wide concentration range with concentrations of 5.0 – 30.0 µg/mL was made showing good correlation with r2 of 0.9992. All other calibration curve points were constructed using six points achieving suitable correlation coefficients (Table 2) and the linearity of the proposed method for valganciclovir was accessed by calculating slope, intercept and correlation coefficient [r2] of standard curve. These results showed that there was an excellent correlation between the peak area and analyte concentration. The slope and intercept of the calibration plot of valganciclovir was 99955.0343x+169015.06 respectively.

iv. LOD and LOQ: The LOD and LOQ values for valganciclovir were 0.805 and 2.685 respectively. The results of LOD and LOQ were presented in Table 2 respectively which revealed the sensitivity of the developed RP-HPLC method**.**

v. Precision: The precision of the current RP-HPLC method was assessed by six replicate injections of 100 % test concentration and the results were expressed in terms of standard deviation and % RSD. The % RSD values for the peak areas of valganciclovir in the study of system precision were found to be 0.0991 and 1.011 respectively and these results indicated that the current RP-HPLC method was highly precise. The system precision results were given in Table 3.

vi. Accuracy: The accuracy of the method was determined by standard addition method that was performed at three concentration levels of 50 %, 100 % and 150 %. The standard drug solutions

were analyzed in triplicate at each level as per the proposed method and the percent recovery at each level was calculated and results are presented in Table 4. These results showed the best recovery i.e. 99.85 - 99.96 % for valganciclovir, indicating the developed RP-HPLC method was accurate.

vii. Robustness: The robustness of the developed method was done by altering the experimental conditions that includes effect of change in flow rate and column temperature. The flow rate of the mobile phase in the current assay was 1.2 mL/min. To study the effect of the flow rate on the resolution, the flow rate was changed by 0.2 units (1.0 and 1.4 mL/min). The effect of the column temperature on the resolution was studied at 33°C and 37°C instead of 35°C. In all the above said varied conditions, the components of the mobile phase were remained constant.

The results of robustness study of the developed assay method are presented in Table 5. These results showed that during all variance conditions, assay value of the test preparation solution was slightly affected and was in accordance with that of actual. The system suitability parameters were also found satisfactory and hence the developed method was concluded as robust.

viii. Ruggedness: The ruggedness of the proposed RP-HPLC method was evaluated by two different analysts with different instruments in the same laboratory. The % RSD for peak areas of valganciclovir was calculated and the experimental results are shown in Table 6. These results revealed that the % RSD was within the limits indicating that the developed RP-HPLC method was found to be rugged.

ix. Solution Stability Study: The stability studies on valganciclovir (10 μgmL^{-1}) in mobile phase were carried out for 24hrs at 35ºC. From these results it is revealed that valganciclovir was stable in mobile phase at least for 24hrs that indicated reliability of analysis in the proposed procedure (Table 7).

x. Analysis of Pharmaceutical Formulations: Analysis of marketed tablets (VALCYTE) was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for valganciclovir (Figure 3**)** was found to be 99.99 % respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95 % to 100 %. The results are given in Table 8.

CONCLUSION

The developed method for the determination of valganciclovir using phosphate buffer and acetonitrile in the ratio of 30:70 v/v at a flow rate of 1.2 mL/min as mobile phase and UV detection at 254 nm had resulted in the elution of the above mentioned two drugs at low retention time than the earlier reported methods (i.e. 3.414 minutes for valganciclovir). The system suitability parameters are within the acceptance criteria. The regression equation obtained for valganciclovir was y = 99955x + 169015 with correlation coefficient r2=0.9992 revealing that the developed RP HPLC method is linear. The assay results (n=6) for method precision and accuracy of valganciclovir were found to be within the ICH limits revealing that precision of the developed method.

The low % RSD values of robust and rugged studies revealed that proposed method is robust and rugged. The proposed method was also subjected to degradation studies which showed that the method was a stability indicating method. From the results of complete validation data it is concluded that "the proposed RP-HPLC method is sensitivity, precise, economical and reproducible making the proposed method applicable for the analysis of valganciclovir in dosage forms in analytical laboratories".

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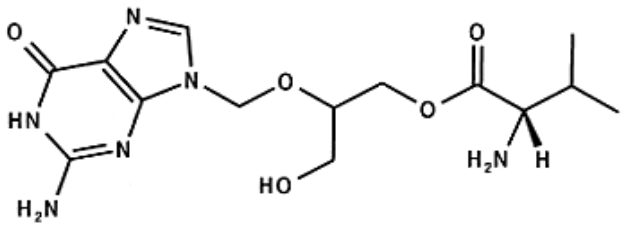
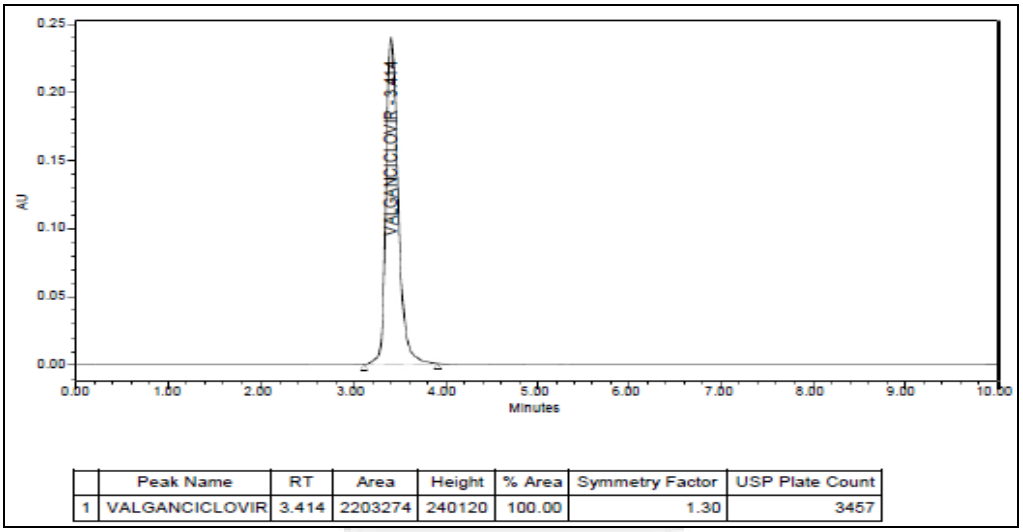
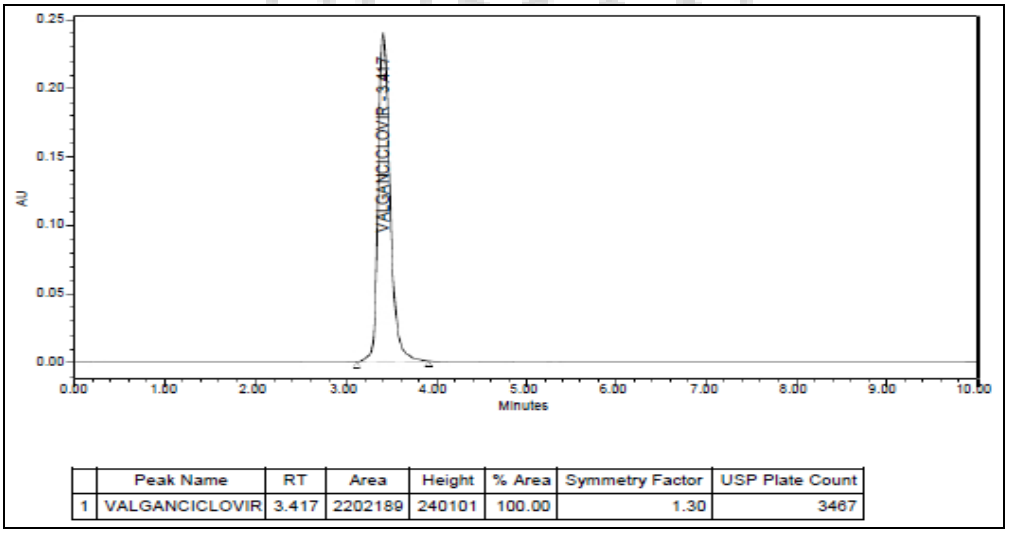


Figure 1. Molecular Structure of Valganciclovir



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	VALGANCICLOVIR	3.414	2203274	240120	100.00	1.30	3457

Figure 2. Typical HPLC Chromatogram Showing the Peak of Valganciclovir



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	VALGANCICLOVIR	3.417	2202189	240101	100.00	1.30	3467

Figure 3. Chromatogram of Valganciclovir in Formulations

Table 1: System Suitability Parameters for Valganciclovir by the Proposed RP-HPLC Method

Name of the Compound	Retention Time	Theoretical Plates	Tailing Factor	Peak Area
Valganciclovir	3.414	3457	1.30	2203274

Table 2: Linearity Studies of Valganciclovir by the Proposed Method

Linearity of Response for Valganciclovir		
% Level (Approx.)	**Concentration (µg/Ml)**	**Peak Area Ratio**
25	5	673279
50	10	1132298
75	15	1689555
100	20	2203274
125	25	2656557
150	30	3154406
Slope, b		99955.0343
Intercept, a		169015.067
r2		0.9992
LOD (µg/mL)		0.805
LOQ (µg/mL)		2.685

Table 3: Results of Method Precision by the Proposed Method

Sr. No.	Name	Rt	Area
1	SOLUTION-1	3.410	2289564
2	SOLUTION-2	3.415	2301243
3	SOLUTION-3	3.413	2311084
4	SOLUTION-4	3.413	2278452
5	SOLUTION-5	3.418	2272103
6	SOLUTION-6	3.419	2246075
AVG*		3.414	2283087
STD DEV*		0.003386	23097.37
% RSD*		0.0991	1.011

*Average of six determinations considered

Table 4: Accuracy Results of Valganciclovir

Sr. No.	50% Area	100% Area	150% Area
INJECTION-1	1101762	2200563	3273763
INJECTION-2	1101081	2400450	3443340
INJECTION-3	1101023	2193630	3443130
AVG*	1101288	2264881	3386744
AMT. RECOVERED*	49.98	99.85	149.95
% RECOVERY*	99.96	99.85	99.96

*Average of three determinations considered

Table 5: Results of Robustness Study

Robust Conditions		Valganciclovir		
		Theoretical Plates	Rt	Peak Area
Flow Rate	1.0 ml/min	3445	3.414	2213265
	1.4 ml/min	3475	3.364	2210564
Temperature	33°C	3432	3.512	2227645
	37°C	3479	3.330	2192476

Table 6: Results of Ruggedness Studies of Valganciclovir

Sr. No.	Name	Analyst-1	Analyst -2
		Area	Area
1	INJECTION-1	2289564	2178364
2	INJECTION-2	2301243	2179078
3	INJECTION-3	2311084	2190183
4	INJECTION-4	2278452	2231473
5	INJECTION-5	2272103	2212746
6	INJECTION-6	2246075	2210343
AVG*		2283087	2200365
STD DEV*		23097.37	21266.93
% RSD*		1.011	0.996

*Average of six determinations considered

Table 7: Stability of Capecitabine in Mobile Phase at Concentration of 10 µg/ml

Mean±SD Concentration of Capecitabine in Mobile Phase at 35 ºC (N=6)					
At 0hr	**At 2hrs**	**At 6hrs**	**At 12hrs**	**At 20hrs**	**At 24hrs**
99.98±0.77	99.73±0.38	100.01±0.55	100.03±0.28	99.86±0.64	99.92±0.58

Table 8: Analysis of Marketed Tablets

Drug	Label claim	Quantity found*	% Assay
Valganciclovir	**450 mg**	**449.99**	**99.99**

*Average of six determinations considered