Available Online at

http://www.ijcpa.in

January-March 2020

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726; pISSN: 2395-2466

DOI: http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-7 | Issue-2 | Article ID: 0032

NEW UV SPECTROPHOTOMETRIC METHOD FOR DEVELOPMENT AND VALIDATION OF VALGANCICLOVIR HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Karthik K.*, Jose Gnana Babu C., Sowyma H. G.

Department of Pharmaceutical analysis, Bharathi College of Pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya district-571422, India

***Corresponding Author:** Email: karthik11201243@gmail.com*

Received: 22 January 2020 / Revised: 20 March 2020 / Accepted: 22 March 2020 / Available online 31 March 2020

ABSTRACT

A simple, sensitive, and economical UV spectrophotometric method has been developed for the estimation of Valganciclovir in bulk and pharmaceutical dosage form. Validation study was performed to develop a simple, sensitive, rapid, accurate, and economical UV spectrophotometric method for the estimation of Valganciclovir hydrochloride. UV 1800 double beam UV visible spectrophotometer with the pair of 10 mm path length matched Quartz cells were used for the study. Valganciclovir shows the maximum absorbance at 252 nm. Hence the area under the curve was measured at 247 nm and 257 nm, respectively. The linearity was measured at the concentration range of 1-6 µg/ml with the correlation coefficient of 0.9994. The regression equation was found to be Y=0.0354 x-0.0041. The standard plot clearly shows a straight line passing through the origin. The limit of detection and the limit of quantitation were found to be 0.0522 µg/ml and 0.1589 µg/ml, respectively. The results of analysis were validated according to the ICH guidelines and by recovery studies are found to be satisfactory. The above method was applied to pharmaceutical dosage forms successfully.

Keywords – *Valganciclovir hydrochloride, UV visible spectrophotometer, Area under curve, Recovery studies, ICH guidelines.*

1. INTRODUCTION

Valganciclovir hydrochloride is a hydrochloride salt form of Valganciclovir, a prodrug form of ganciclovir, a nucleoside analogue of 2'-deoxyguanosine, with antiviral activity. After the completion of phosphorylation, Valganciclovir is incorporated into DNA, resulting in the inhibition of viral DNA polymerase, and viral replication. Valganciclovir hydrochloride is an antiviral agent that is used to treat cytomegalovirus retinitis in patients with AIDS, and for the prevention of cytomegalovirus infections in organ transplant recipients who have received an organ from a CMV-positive donor. The Valganciclovir acts by slowing the growth of the CMV virus. It helps prevent the spread of infection to other areas of the body.

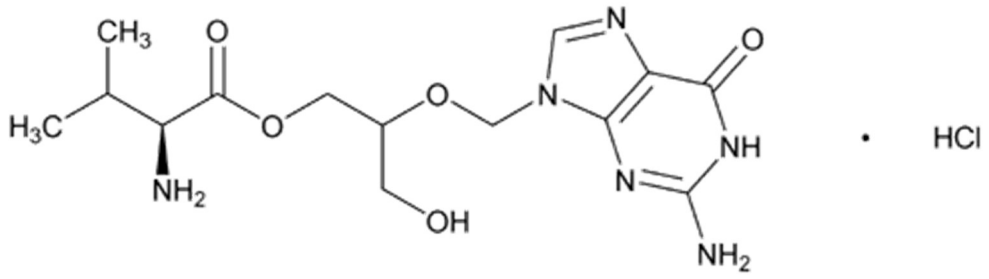

Fig. 1: Chemical structure of Valganciclovir hydrochloride[1]

Valganciclovir hydrochloride contains not less than 97% and not more than 102%. Hydrochloride is calculated based on the anhydrous and solvent free basis. Valganciclovir hydrochloride is available in the form of White to off crystalline powder. The crystals from water+isopropranol undergo phase changes at 142˚C. It is freely soluble in Water, Dimethyl sulfoxide, Methanol, Acetonitrile and Acetic acid. Valganciclovir hydrochloride is available under the brand name of Valcyte, Cymeral, Rovalcyte and Darilin [2-6].

Literature Survey revealed that the drug has been estimated by UV-Spectrophotometric[7-13], RP-HPLC[14-17], HPTLC[18] and Liquid chromatographic method[19-21] has been reported so far.

The present study describes a simple, precise and accurate analytical method for the estimation of Valganciclovir hydrochloride in bulk and pharmaceutical dosage forms. The above method was developed and validated according to the ICH guidelines.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The Valganciclovir hydrochloride was obtained as a gift sample from pharma industry. All the chemicals were of under analytical grade. The tablet formulation was procured from local pharmacy store. Distilled water was used throughout the analytical method development.

2.2 Instrumentation

UV 1800 double beam UV spectrophotometer with a pair of 10 mm path length of two quartz cells were used for the study.

2.3 Preparation of standard stock solution

Weigh accurately about 10 mg of Valganciclovir hydrochloride and transferred into 100 ml clean and dried volumetric flask and make up the volume up to 100 ml with Distilled water (100 µg/ml). From above solution carefully pipette out 10 ml of solution and transferred into 100 ml clean and dried volumetric flask and make up the volume up to 100 ml with Distilled water (10 µg/ml). Above standard solution was prepared and scanned in the range of 200-400 nm against Distilled water as blank. It shows the maximum absorbance at 247 nm and 257 nm respectively (figure 2).

2.4 Preparation of calibration curve

Aliquots of a solution 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml (10 µl/ml) were transferred to 10 ml volumetric flasks marked as A, B, C, D, E and F respectively diluted up to the mark using distilled water. The absorbance is measured at 247 nm and 257 nm against reagent as distilled water. The calibration curve was plotted between the concentration of Valganciclovir hydrochloride and the measured absorbance. It has shown in the figure 3. The data is obtained statistically to calculate the coefficient of correlation, linearity, range, slope and intercept.

2.5 Accuracy

For assay methods, samples are prepared in triplicate at three concentration levels covering the specified three levels over a range of 50–150 % of the target concentration.

2.6 Precision

2.6.1 Intraday Precision: It is determined by analyzing the drug at a 6 different concentration and each concentration for six times on a same day and calculated the value of Mean, SD, and % RSD.

2.6.2 Inter day Precision: It is determined similarly, but the analysis being carried out daily for six consecutive days and calculated the value of Mean, SD, and % RSD

2.7 Limit of Detection and Limit of Quantification

Limit of Detection and Limit of Quantification are two important performance characteristics in method validation. The LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. The LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10 σ/S criterions, respectively, where σ is the standard deviation of the S-intercepts of the regression lines and σ is the slope of the calibration curve.

2.8 Preparation of Sample solution

The marketed tablet formulation of Valganciclovir was analysed by the proposed method. Weigh about 20 tablets of Valganciclovir hydrochloride and grind to fine powder by using mortar and pestle. From this weigh about 10 mg of powder was transferred into a 100 ml volumetric flask. Weighed powder was dissolved in 50 ml of distilled water and shaken for 15 minutes. The solution was filtered through Whatman filter paper no. 40 into 100 ml volumetric flask and diluted with the same solvent to get the concentration within the linearity. The absorbance was measured at 247 nm and 257 nm respectively and the concentration was determined from regression equation of calibration curve.

2.9 Method Validation

The method is validated according to the ICH guidelines Validation methods like Linearity, Precision, Accuracy, Ruggedness, LOD and LOQ was carried out according to the ICH guidelines.

All the tablet formulations contained different excipients and binders, which were added to the formulations along with the active pharmaceutical ingredients. These substances cause some interference during estimation of the active drug constituents. And the Interferences from the excipients were confirmed by performing the recovery experiments for which standard addition method was performed. So in order to ensure the accuracy and reproducibility of the results obtained, recovery studies were carried out by the addition of known amount of standard drug solution of Valganciclovir hydrochloride to pre-analyse the tablet sample solution at three different concentration levels within the range of linearity. The resulting mixtures were analysed by the proposed method. The data results obtained for the determination of Valganciclovir in tablet formulation by the proposed method. It is shown in Table no 2. Also, the experiment was repeated 6 times in a day to determine intraday precision and on the 6 different days to determine interday precision. The percentage Relative standard deviation (%RSD) was calculated at each concentration level.

3. RESULTS AND DISCUSSION

The development of new formulation or dosage form involves a number of stages and procedures and the analytical methods that are specific, accurate and precise plays a very important role in many of the essential features that are useful for an identical analytical system. An accurate, economical and rapid spectrophotometric method was developed for the quantitative estimation of Valganciclovir hydrochloride in bulk and tablet dosage forms. The linearity range of Valganciclovir hydrochloride was determined in Distilled water and found to be 1-6 µg/ml. It is shown in the table no 1. Commercial formulations (tablet) containing Valganciclovir hydrochloride was successfully analysed by the proposed method. S.D. values were low that indicated reproducibility of the proposed method. The accuracy, recovery experiments were also performed by adding known amount of free drug to previous analysed by the proposed method. The results are summarized in Table 4. The percentage of drug recovered 99.39 -100.4% was in good agreement with the added amount and labelled claim indicating reproducibility of the methods. Precision was performed by two ways interday and intraday precision. The results obtained are satisfactory and the %RSD values are low well within the range of ICH guidelines. Results are shown in the table no 3. Ruggedness was performed by the different analysts. The %RSD values are low well within the range of ICH guidelines. Results are shown in the table no. 5.

Table 1: Results of calibration curve at 247-257 nm by Area under curve.

Sr. No.	Concentration	Absorbance
1	1µg/ml	0.033
2	2 µg/ml	0.066
3	3 µg/ml	0.10
4	4 µg/ml	0.137
5	5 µg/ml	0.175
6	6 µg/ml	0.208

Table 2: Regression parameters of Valganciclovir hydrochloride by Area under curve

Sr. No.	Regression Parameters	Value
1	Beer's law Range	1-6µg/ml
2	λMax	247-257nm
3	Regression Equation	Y=0.0354x-0.0041
4	Slope (b)	0.0354
5	Intercept(a)	0.0041
6	Correlation coefficient (r2)	0.9994
7	Sandell's Sensitivity	0.0294
8	Molar extinction co-efficient	3.4×10^{-4}
9	Limit of Detection	0.0522µg/ml
10	Limit of quantitation	0.1589µg/ml

Table 3: Results of precision for Valganciclovir hydrochloride at 252 nm by Area under curve

Concentration (µg/ml)	Intra-day Absorbance ± SD**	% RSD	Inter-day Absorbance ±SD**	%RSD
1	0.0335±0.0005	1.49	0.0335±0.0005	1.49
2	0.0655±0.00125	1.83	0.066±0.00057735	0.86
3	0.1015±0.00160	1.58	0.101±0.001527525	1.5
4	0.1355±0.00170	1.25	0.1366±0.001105542	0.8
5	0.1768±0.00203	1.13	0.1766±0.001972027	1.07
6	0.2083±0.00221	1.05	0.2083±0.001374369	0.65

Table 4: Results of accuracy for Valganciclovir hydrochloride at 252nm by Area under curve

Spiked levels	Amount of sample (µg/ml)	Amount of Standard (µg/ml)	Amount recovered	% Recovery ±SD**	% RSD
50	3	4.5	4.48	100.4±1.491	1.48
100	3	6	5.94	99.18±0.150	0.15
150	3	7.5	7.48	99.39±1.675	1.68

**Average of six determinations

Table 5: Results of Ruggedness for Valganciclovir hydrochloride at 252 nm by Area under curve

Analysts	Analyst-1	Analyst-2	Analyst-3	Mean absorbance ±SD**	%RSD
1	0.034	0.033	0.033	0.0333±0.000577	0.000577
2	0.066	0.064	0.066	0.0653±0.001155	0.001155
3	0.102	0.099	0.1	0.1003±0.001528	0.001528
4	0.136	0.135	0.137	0.136±0.001	0.001
5	0.178	0.176	0.175	0.1763±0.001528	0.001528
6	0.21	0.207	0.208	0.2083±0.001528	0.001528

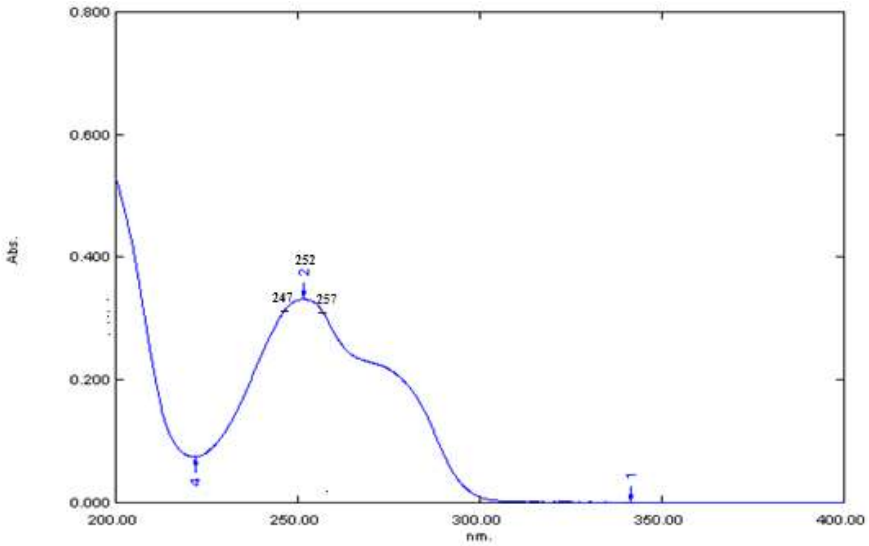

Fig. 2: Area under curve spectra of Valganciclovir hydrochloride showing the absorbance from **247nm to 257nm.**

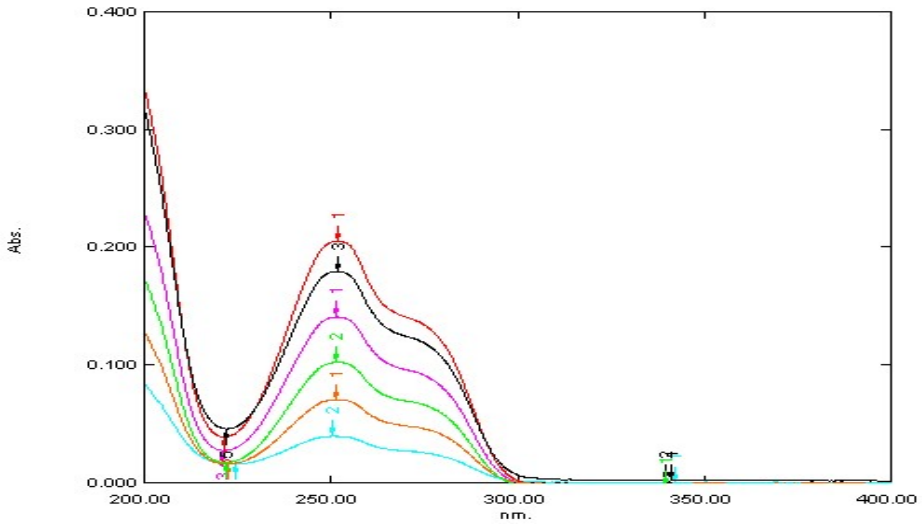

Fig. 3: Area under curve overlain spectra of Valganciclovir hydrochloride showing the absorbance from 247nm to 257nm.

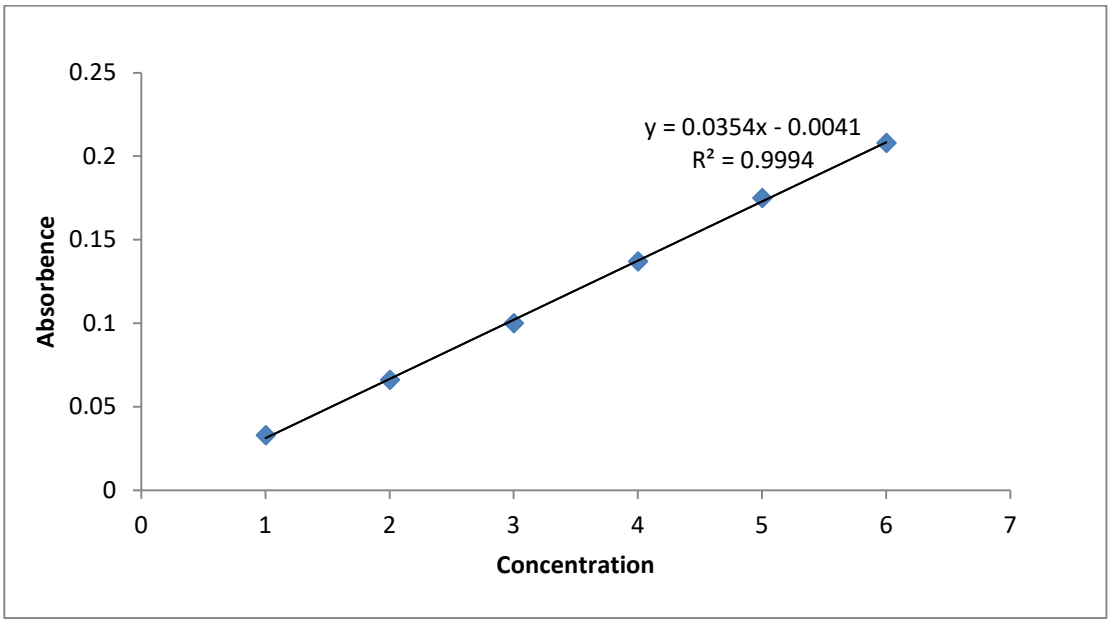

Fig. 4: Linearity curves for Valganciclovir hydrochloride by Area under curve

4. CONCLUSION

From the above it can be concluded that all validation parameters such as precision, accuracy, linearity, LOD, LOQ and Ruggedness met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed spectrophotometric method is simple, rapid, accurate, and precise and can be applied for routine analysis of Valganciclovir hydrochloride in bulk and its dosage forms.

5. ACKNOWLEDGEMENT

We would like to thanks to Management, Principal, Teaching staff, non-teaching staff and my dear Friends of Bharathi College of pharmacy for their continues co-operation and support.

REFERENCES

1. https://www.drugfuture.com/Pharmacopoeia/USP32/pub/data/v32270/usp32nf27s0_m87581.html
2. https://www.drugbank.ca/drugs/ Valganciclovir hydrochloride.
3. https://www.webmd.com/drugs/2/drug-20977/ Valganciclovir-oral/details
4. Porter SR, Kaplan JL. Merck index manual of diagnosis and therapy. 19th ed. Merck sharp & Dohme corp: New Jersey; 2011.
5. O'Neil JM, Heckelman EP, Dobbelaar HP, Roman JK, Kenny MC, Karaffa SL. The merck index an encyclopedia of chemicals, drugs and biological, 15thed. Royal society of chemistry: United Kingdom; 2013.
6. U.S Pharmacopoeia-National Formulary [USP 34 NF 29]. Vol III. Baltimore. Rockville Md: United States pharmacopoeial convention, United book press; 2011.
7. Kar A. Pharmaceutical drug analysis. 2thed. New ageinternational private limited: New Delhi; 2005.
8. Sharma BK. Instrumental method of chemical analysis. 23rded. Goel publishing house: Meerut; 2004.
9. Willard HH, Merritt LL, Dean JJA, Frank AS. Instrumental method of analysis: 23rded. CBS publishers and Distributers: New Delhi; 1986.
10. Michael E, Schartz IS, Krull. Analytical method development and Validation: 2004.

11. Dasari V, Chandu BR, Khagga M, Gindi S. New simple UV spectrophotometric method for the estimation of Valganciclovir in bulk and its formulation. Int J Adv Pharm sci 2010;1(2):282-6.
12. Mondal S, Reddy GS, Mondal P, Prathyusha VS, Nair AP, Rahaman ST. Development and Validation of Few UV Spectrophotometric Methods for the Determination of Valganciclovir in Bulk and Pharmaceutical Dosage Form. J Pharm Anal Pharm 2018;9(2):64-8.
13. Awen BZ, Dassari V, Chandu BR, Khagga M, Katakam P. New simple UV Spectrophotometric method for the estimation of Valganciclovir in bulk and its formulation. Int J Pharm Res 2011;2(1):55-8.
14. Krishna Veni N, Gowramma B, Madhuri L, Gouthami B, Sindhur Nag N, Meyyanathan SN. Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Valganciclovir Hydrochloride (RS). J Pharm Anal 2014;3(1):19-26.
15. Ramesh G, Subba Rao M. Development and Validation of Stability Indicating RP-HPLC Method for Quantitative Determination of Valganciclovir in Pure and Pharmaceutical Formulations. Int J Pharm Pharm Res 2015;3(1):1-14.
16. Sawant S, Barge V. A Validated Stability Indicating RP-HPLC Method for Valganciclovir, Identification and Characterization of forced Degradation Products of Valganciclovir Using LC-MS/MS. Acta Chromatographica 2014;29(1):29-42.
17. Mondal S, Reddy GS, Mondal P, Prathyusha VS, Nair AP, Rahaman ST. A New Stability Indicating Ultra Performance Liquid Chromatography-PDA Method for the Estimation of Valganciclovir in Bulk and Tablet Dosage Form. J Pharm Anal Pharm 2018;9(2):94-8.
18. Surekha ML, Swamy GK. High performance thin layer chromatographic method for determination of Valgacyclovir in tablet dosage form. Bull Pharm Med Sci. 2013;1(1):44-8.
19. Annapurna MM, Tulasi KL, Sirichandra M. Stability indicating Liquid chromatographic method for the quantitative determination of Valganciclovir in pharmaceutical dosage forms J Drug Deliv Ther. 2013;3(3):64-70.
20. Malleswara Rao SN, Srinivas K, Suryanarayana MV, Madhavan P, Mukkanti K. A Validated LC Method for the Determination of Chiral Purity of (S)-2-azido3-methylbutanoic acid: A key Raw material of Valganciclovir hydrochloride. J Chem Pharm Res. 2011;3(4):22-28.
21. Sura S, Modalavalasa RR, Chandra Sekhar KB. Development and Validation of Stability Indicating RP-Liquid Chromatographic Method for the Quantitative Determination of Valganciclovir. Der Pharma Chemica. 2017;9(19):101-109.
22. ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology; 2005.