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Original Article

Development and Validation of Few UV Spectrophotometric Methods for the Determination of Valganciclovir in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Background: The present research paper described about the optimization of various zero order and first order UV spectrophotometric method using different buffers. Validation study was performed to develop a simple, sensitive, rapid, accurate and economical Ultra Violet spectrophotometric method for the estimation of Valganciclovir. **Methods:** UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. Method A (Water), Method B (phosphate buffer pH2), Method C (Phosphate buffer pH4) and Method D (phosphate buffer pH5) were developed for estimation of Valganciclovir by zero-order and first-order derivative. **Results:** Linearity was carried out in the concentration range of 5-60 µg/ml and the correlation coefficient were found to be 0.999. The percentage recoveries were found to be 98-102%. The relative standard deviation was found to be <2%. The LOD and LOQ were found to be 0.3241 µg/ml and 0.8227 µg/ml respectively. **Conclusion:** Hence, the methods were validated according to ICH guidelines and can be adopted for the routine analysis of Valganciclovir in pure and tablet dosage form.

Key words: Valganciclovir, UV Visible Spectrophotometer, Zero-order, First-order derivative, ICH guidelines.

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INTRODUCTION

Valganciclovir (VGC), chemically L-Valine, 2[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-3-hydroxypropyl ester, monohydrochloride. (Figure 1) is a white to off-white crystalline powder with a molecular formula of $C_{14}H_{22}N_6O_5$·HCl and a molecular weight of 390.83. It is an antiviral medication used to treat cytomegalovirus infections. It is actually a prodrug for ganciclovir. After oral administration, it is rapidly converted to ganciclovir by intestinal and hepatic esterases. The mechanism of action of VGC is that it is a prodrug of ganciclovir that exists as a mixture of two diastereomers. After administration, these diastereomers are rapidly converted to ganciclovir by hepatic and intestinal esterases. In cytomegalovirus infected cells, ganciclovir is initially phosphorylated to the monophosphate form by viral protein kinase and then it is further phosphorylated via cellular kinases to produce the triphosphate form.[1]

Very few methods are reported in the literature including liquid chromatographic methods,[2-4] spectrophotometric techniques[5] and LC/MS/MS[6-8] methods for the determination of Valganciclovir in tablet dosage forms

As there was no work in the literature about the UV spectrophotometric method for the analysis of VGC using method a, b, c and d by zero order and first order derivative. Therefore, the objective of present study was to develop and validate a simple, precise, accurate and economical UV spectrophotometric method for estimation of VGC.

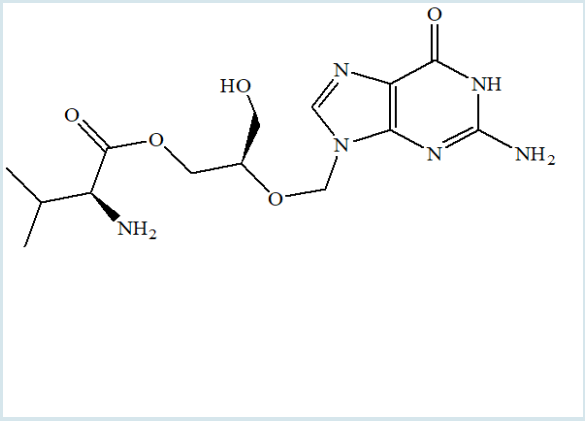

Figure 1: Chemical structure of Valganciclovir

MATERIALS AND METHODS

Chemicals and reagents

Valganciclovir was obtained as a gift sample from Mylan Laboratories Ltd., Hyderabad. All the chemicals used were of analytical grade. The tablet formulations were procured from local pharmacy. Distilled water was used throughout the experiment

Instrumentation

UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used.

Preparation of standard drug solution

Standard stock solution of 100 µg/ml was prepared by dissolving accurately weighed 10 mg of VGC in 50 ml methanol in 100 ml volumetric flask and sonicated for 10 mins. The final volume was adjusted to 100 ml with methanol. The prepared standard solution was scanned in the range of 200-400 nm to determine the wavelength of maximum absorption.

Validation Procedure

The proposed method was optimised using methanol as stock solvent. Water (Method A) and various buffers (method B, C and D) as diluted solvents. The present method was validated for the various parameters as per ICH guidelines.[9]

Linearity

Different aliquots were taken from working solution and diluted with Water, (Method A) phosphate buffer pH-2 (Method B), pH-4 (Method C), pH-5(Method D) separately to prepare series of concentrations from 5-60 µg/ml.[10] Absorbance was measured at 252.5, 252, 252, 252 nm. Finally, the calibration curve was plotted between concentration and absorbance.

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.[11]

Precision

Intraday Precision

It is determined by analyzing the drug at a 3 different concentration and each concentration for three times on a same day and calculated the value of Mean, SD, and %RSD.

Inter day Precision

It is determined similarly, but the analysis being carried out daily for three consecutive days and calculated the value of Mean, SD, and %RSD.

Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal to- noise ratio and the use of standard deviation of the response and the slope of the calibration curve. 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.

Assay of marketed dosage form

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to10 mg of VGC was transferred to 100 ml volumetric flask and dissolved in methanol and final volume was made up with the same. The sample solution was then filtered through Whatman filter paper no. 41. This is stock solution of 100 ug/ml. From the above stock solution 0.5, 1, 2, 3, 4, 5, 6, ml of solution was transferred in 10 ml volumetric flask and was diluted with methanol upto 10 ml. This gives solution of 5 to 60 ug/ml concentration of VGC. These solutions were scanned under entire uv region (400 nm to 200 nm) and area of it between the wavelength range 252 nm to 254 nm was calculated to get the concentration of drugs.

RESULTS

The standard solutions of VGC in methanol (10 µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm at first order derivative mode and the first order derivative spectra was taken at smoothening factor of the instrument using Shimadzu 1800 spectronic UV Visible spectrophotometer. Absorption maximum of VGC was found to be at 251-253 nm. Overlain spectra were depicted in Figure 2-5 and summary of validation parameters were represented in Table 1

Table 1: Summary of validation parameters

Methods	Paramaters			
	Correlation coefficient	%Recovery ± SD	Sandell's sensitivity	Molar absorptivity
Method A				
i)zero order	0.9992	101.7±0.26	0.0278	13087.88
ii)first-order derivative	0.9993	99.04±0.90		
Method B				
i)zero order	0.9992	99.6±1.56	0.0299	12184.23
ii)first-order derivative	0.9991	98.5±1.1		
Method C				
i)zero order	0.9993	99±0.92	0.0361	11514.78
ii)first-order derivative	0.9994	99.98±1.43		
Method D				
i)zero-order	0.9997	99.92±1.76	0.0322	11296.15
ii)first-order derivative	0.9995	98.7±1.70		

Table 2: Assay of marketed formulation using various methods.

Methods	Labeled claim(mg)	Drug recovered (mg)	%Drug recovered ±SD	%RSD
A	450	445.63	98.88±0.39	0.52
B	450	446.92	99.31±0.41	0.94
C	450	446.56	99.23±0.82	0.83
D	450	446.43	99.20±0.49	0.59

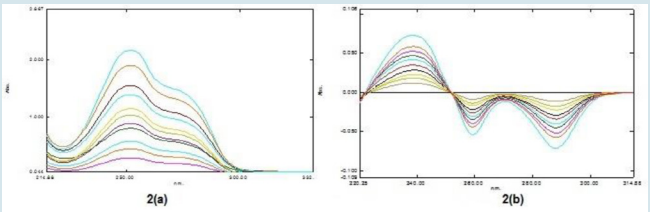

Figure 2(a): Overlain spectra of method A (Zero-order).
Figure 2 (b): Overlain spectra of method A (first-order)

The assay of the marketed dosage form was performed by using the developed method and percentage purity was found 98.88, 99.31, 99.23 and 99.20 respectively which is found within the acceptable purity level as per ICH guidelines. The details of the result shown in Table 2.

Beer Lambert's law was obeyed in the concentration range of 5-60µg/ml. The R^2 value was found to be greater than 0.999. Therefore, which is within the limits, hence there is a good linear relationship between concentration and absorbance. Calibration curves were shown in Figure 6-9.

Accuracy for the methods was established at 50, 100, 150% levels by the addition of standard drug of Valganciclovir to the pre-quantified sample solution. Each dilution was observed three times and the percentage recovery of the drug was measured and the mean recovery was found to be in the range of 98% -102%. The %RSD values were found to be <2, therefore which is within the limits and it indicates that the method was accurate and the results were given in the Table no. 2-5.

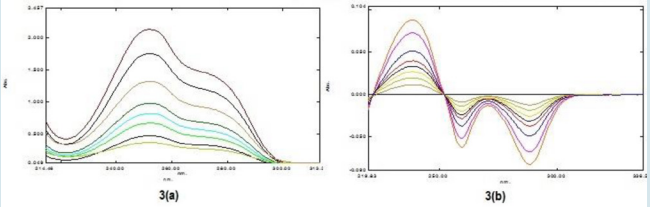

Figure 3(a): Overlain spectra of method B (Zero-order).
Figure 3 (b): Overlain spectra of method B (first-order).

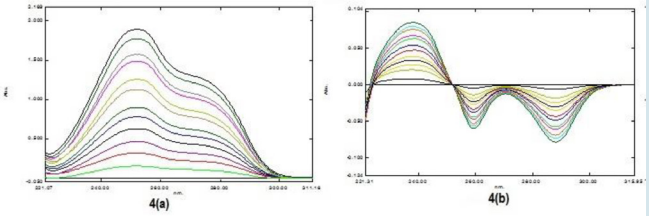

Figure 4. (a): Overlain spectra of method C (Zero-order).
Figure 4 (b): Overlain spectra of method C (first-order).

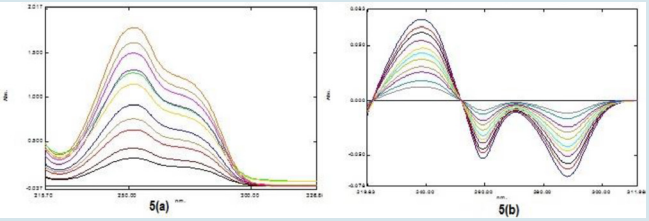

Figure 5. (a): Overlain spectra of method D (Zero-order).
Figure 5(b): Overlain spectra of method D (firstorder).

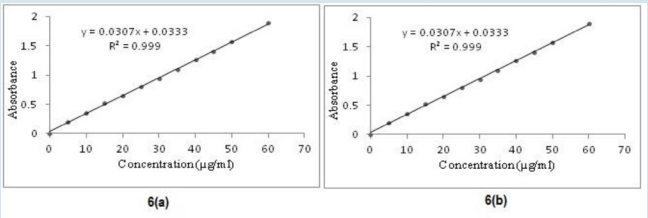

Figure 6. (a): Calibration curve of method A (Zero-order derivative).
Figure: 6 (b) Calibration curve of method A (first-order derivative).

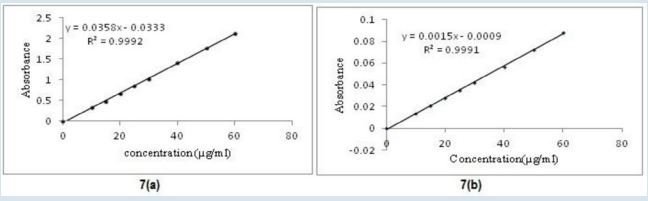

Figure 7. (a): Calibration curve of method B (Zero-order derivative).
Figure: 7 (b) Calibration curve of method B (first-order derivative).

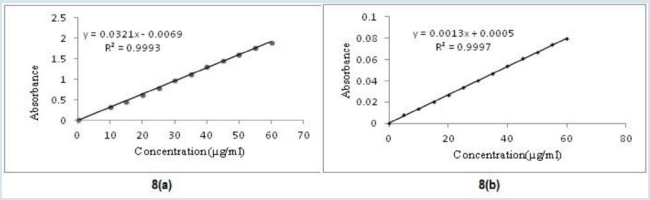

Figure 8. (a): Calibration curve of method C (Zero-order derivative).
Figure:8 (b) Calibration curve of method C (first-order derivative).

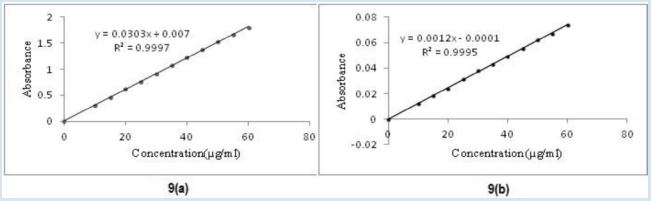

Figure 9. (a): Calibration curve of method D (Zero-order derivative).
Figure:9 (b) Calibration curve of method D (first-order derivative).

Table 2: Accuracy data of method A.

Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	5	15.25	101.7 ± 0.26
	10	10	19.99	99.77 ± 0.25
	10	15	25.3	101.1± 0.53
First order	10	5	14.75	99 ± 0.9
	10	10	19.6	98.4 ± 1.02
	10	15	24.6	98 ± 0.36

Table 3: Accuracy data of method B.

Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	5	15	99.6 ± 0.78
	10	10	20.11	100.7 ± 0.56
	10	15	25.25	100.7 ± 0.31
First-order	10	5	14.9	98.5±1.1
	10	10	19.4	98.9±0.50
	10	15	24.3	98.2±0.60

Table 4: Accuracy data of method C.

Method C	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	5	14.74	99±0.93
	10	10	20.16	100.3±1.47
	10	15	24.62	98.51±0.44
First order	10	5	15	99.98±1.43
	10	10	19.7	98.8 ± 0.92
	10	15	24.7	98.6 ± 0.43

Table 5: Accuracy data of method D.

Method D	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	5	14.95	99.92±1.76
	10	10	20.06	100.93±0.65
	10	15	24.98	100±1.50
First order	10	5	14.75	98.7±1.7
	10	10	19.8	99.8±0.70
	10	15	24.9	100.07±0.50

Precision of the method was studied by repeated measurements of drug solution and results showed lower %RSD values. The %RSD values were found to be ≤2. Therefore, which is within the limits and it indicates that the method was precise and the %RSD for intra-day precision and inter-day precision for Valganciclovir were shown in Table 6-13.

LOQ is defined as lowest amount of analyte which can be detected. LOD was defined as lowest amount of analyte which can be quantitatively determined.LOD and LOQ of the drug were calculated as per ICH guidelines. The LOD and LOQ for Valganciclovir were found to

Table 6: Intraday precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	15.1	101.8± 0.10	0.11
	30	29.72	99.06±0.13	0.12
	60	60.30	101.2±0.22	0.23
First order	15	14.9	99.22±1.83	1.85
	30	29.8	99.46±1.67	1.67
	60	60.3	100.22±0.79	0.79

Table 7: Inter day precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	15.31	101.9± 0.14	0.13
	30	29.71	99.01±0.08	0.09
	60	60.97	100.95±0.12	0.13
First order	15	15	99.14±1.45	1.46
	30	29.9	99.44±1.68	1.69
	60	59.9	100.62±1.40	1.39

Table 8: Intraday precision data of method B.

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	14.85	99.93± 0.80	0.80
	30	30.02	98.69±1.31	1.30
	60	60.97	99.25±1.88	1.89
First order	15	14.6	101.11±1.02	1.01
	30	30.3	101±0.51	0.50
	60	60.2	100.9±0.54	0.53

Table 9: Inter day precision data of method B.

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	14.94	98.76± 0.79	0.80
	30	29.95	98.97±1.20	1.21
	60	60.02	98.93±1.91	1.92
First order	15	14.9	99.33±1.68	1.68
	30	30.4	100.44±0.84	0.84
	60	60.9	100.83±0.67	0.66

Table 10: Intraday precision data of method C.

Method C	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	14.90	99.04± 0.87	0.88
	30	30.41	101.2±0.42	0.41
	60	59.42	98.85±0.19	0.19
First order	15	14.8	100.4±1.68	1.67
	30	30.1	101.3±0.88	0.87
	60	60.1	100.8±0.59	0.59

Table 11: Inter day precision data of method C.

Method C	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	14.74	98.28± 0.64	0.65
	30	30.51	101.9±0.21	0.20
	60	59.35	98.69±0.21	0.21
First order	15	15.2	99.78±1.68	1.68
	30	30.2	100.7±1.34	1.33
	60	60.9	101.1.±0.44	0.53

Table 12: Intraday precision data of method D.

Method D	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	15.21	101.4± 0.68	0.67
	30	30.42	100.9±0.46	0.45
	60	59.66	99.43±0.23	0.23
First order	15	14.9	99.77±1.39	1.39
	30	29.6	100.55±1.65	1.64
	60	60.2	100.11±1.02	1.02

Table 13: Inter day precision data of method D.

Method D	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	15	14.95	99.73± 0.57	0.57
	30	30.03	99.80±0.27	0.26
	60	60.04	99.21±0.19	0.20
First order	15	14.9	98.89±1.39	1.40
	30	30.02	99.85±0.82	0.80
	60	60.4	100.16±1.48	1.49

0.3241µg/ml and 0.8227µg/ml respectively. Hence it shows the proposed method was found to be sensitive.

DISCUSSION

The objective of the analytical procedure is to govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Linearity, Accuracy, Precision, LOD and LOQ. The linear regression data for the calibration plot were indicative of a good linear relationship between peak area and concentration over wide range. The result shown that best recoveries (98-102%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Precision of valganciclovir was evaluated and the percentage relative standard deviation (%RSD) was found to be less than 2% which proves that the method was precise. And the Limit of Detection and Limit of Quantification was found to be 0.3241µg/ml and 0.8227µg/ml respectively. Hence the proposed method was sensitive.

CONCLUSION

The developed methods were validated in terms of linearity, accuracy and precision in accordance with the ICH guidelines. The proposed methods are simple for estimation of Valganciclovir in bulk and dosage form. Validation results are satisfactory and therefore the developed methods can be used for routine analysis of formulations without interference from excipients.

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CONFLICT OF INTEREST

The authors declare no conflcit of interest.

ABBREVIATIONS

VGC: Valganciclovir; **ICH:** International Council for Harmonization; **LOD:** Limit Of Detection; **LOQ:** Limit of Quantitation; **RSD:** Relative Standard Deviation.

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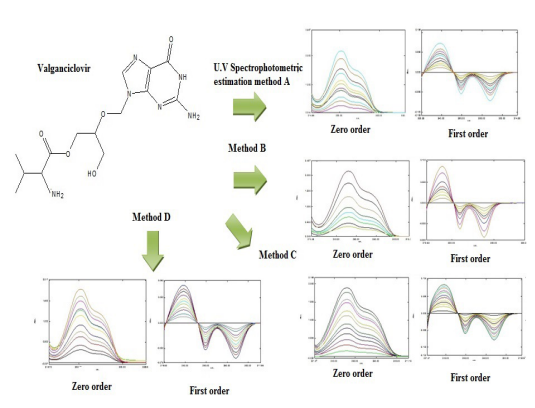
PICTORIAL ABSTRACT



SUMMARY

- Present study was planned to develop and validate a simple, precise, accurate and economical UV spectrophotometric method for estimation of Valganciclovir in Bulk and Pharmaceutical Dosage Form. UV 1800 double beam UV Visible Spectrophotometer were used for the study. The analysis is in 200-400 nm range. The developed method was validated as per ICH guidelines. Validation results are satisfactory and therefore the developed methods can be used for routine analysis of formulations without interference from excipients.

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