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Second-derivative UV spectrometric determination of ganciclovir in its solid dosage form

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ABSTRACT

Ganciclovir is an acyclic guanosine analogue used in the treatment of cytomegalovirus (CMV) infection and AIDS in humans. During the development of Ganciclovir solid dosage form, formulation compositions were constantly varied. A fast and reliable method for the dissolution and release testing of Ganciclovir was highly desirable to support formulation screening. A second derivative UV spectroscopic method was developed for determination of Ganciclovir in the solid dosage form. After carefully choosing a zero-crossing technique of second derivative UV measurement at 253 nm, the selectivity and sensitivity of Ganciclovir was comparable to the previously developed HPLC method. In comparison with the direct UV method, second derivative UV spectroscopy eliminates the interference from UV absorbing excipients, which often results in a standard error of 2–10%. This method is also fast and economical in comparison to the more time-consuming HPLC method regularly used for formulation screening. Finally, this method has been validated to be precise and accurate, and is demonstrated to be an excellent alternative to HPLC method for the dissolution and release testing of Ganciclovir in the solid dosage form.

KEYWORDS

Ganciclovir;
Second derivative UV;
Dissolution;
HPLC;
Release testing.

INTRODUCTION

Derivative UV spectroscopy has been widely used as a tool for quantitative analysis, characterization, and quality control in agricultural[1,2], pharmaceutical[3-6], and biomedical fields. This technique offers various advantages over the conventional absorbency methods such as the discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of overlapping spectra. As a result, derivative spectroscopy usually provides much better fingerprints than the traditional absorbency spectra. Aim of this study was to develop an alternative analytical method, to the more time consuming HPLC method, which can be used regularly and for formulation screening. A second derivative UV spectroscopy was developed to support formulation development of Ganciclovir in a solid dosage form[7].

GANCICLOVIR

GCV or 9-(1,3-dihydroxy-2-propoxy-methyl) gua-

nine (DHPG) (Figure 1), is a nucleoside analogue of guanosine, a homologue of acyclovir, and the first antiviral drug to be effective in the treatment of cytomegalovirus (CMV) infection in humans[8]

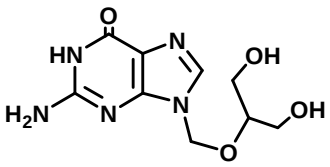

Figure 1 : Chemical structure of Ganciclovir

EXPERIMENTAL METHODS

Apparatus

Spectrophotometric analyses were performed with a computer-based Shimadzu 1800 UV-Visible double beam spectrophotometer. The HPLC analysis was performed on a Jasco 2080 binary gradient chromatograph with Jasco UV detector 2075. Measurements were made with a 10ml injection volume at 45°C; the detector wavelength was set at 253 nm. Routine analyses were carried out in binary gradient system on a Hypersil C-18 column at a flow rate of 1.0 ml/min.

Materials

Ganciclovir capsules (GANGUARD) 500mg were purchased from Ranbaxy Laboratories Limited, Hyderabad.

Methods

(A) Dissolution method conditions

Dissolution USP Apparatus 2 (paddle) with the paddle speed of 60 rpm was used. The capsules were inserted into a plastic coated helical sinker prior to dropping into the dissolution medium which consisted of distilled water with a medium volume of 900 ml and temperature controlled at 37 ± 0.5°C. The sample solutions were directly taken from the dissolution vessel through a syringe and maintained sink condition.

(B) HPLC method for dissolution testing

A Hypersil C 18 column at ambient temperature was eluted with a mobile phase consisting of acetonitrile/water (70:30 v/v) at a flow rate of 1.0 ml/min. Ganciclovir was determined by UV detection at 253 nm. The injection volume was 20 µl and run time was 10 minutes.

(C) Sample solution preparation to content uniformity testing

For release testing, one capsule was placed into each of ten 100 ml volumetric flasks. The volume was diluted with a diluent consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35v/v), and was stirred with a stirring bar for at least 3 h at fast speed until the capsules were dispersed in the solution. Samples were further diluted with 5 ml of the stock solution to 100 ml with the sample diluent consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35 v/v). An aliquot was centrifuged and the supernatant was analyzed.

(D) HPLC method for content uniformity

A Hypersil C-18 column thermostat at 45°C was eluted with a mobile phase consisting of Acetonitrile/ Water (70:30 v/v) at a flow rate of 1.0ml/min. Ganciclovir was determined by UV detection at 253 nm. The injection volume of 20 µl and run time was 10 minutes.

(E) Standard solution preparation

For release testing of Ganciclovir capsules (GANGUARD), 20 mg of Ganciclovir reference standard was accurately weighed into a 100 ml volumetric flask and dissolved in the diluents consisting of acetonitrile/0.025 M phosphate buffer, pH 4.0 (65:35 v/v). The above stock solution (5 ml) was further diluted to 100 ml in a 100 ml volumetric flask to give the standard solution. For dissolution testing, 36 mg of Ganciclovir reference standard was accurately weighed into a 200 ml volumetric flask and dissolved in no more than 10 ml methanol and diluted to 200 ml with a medium consisting of sodium dodecyl sulfate in 0.01 M phosphate buffer, pH 6.8. The above stock solution (25 ml) was further diluted to 200 ml in a 200 ml volumetric flask.

RESULTS AND DISCUSSION

Based upon the direct UV spectroscopic data, there is no wavelength where Ganciclovir can be accurately quantified without substantial background interference. However, the difference do exist between the second derivative UV spectra of Ganciclovir and the excipients in placebo capsules, which indicates the feasibility of a derivative UV method. The second derivative UV spectra of Ganciclovir and the excipients in placebo capsules were subsequently measured. As demonstrated

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in Figure 2 (placebo) and Figure 3 (2nd derivative), Ganciclovir can be measured at 253 nm with little interference in the second derivative mode. The dissolution testing was conducted on Ganciclovir capsule dosage form and the sample solutions were analyzed using direct and second derivative UV spectroscopy followed by currently used HPLC method. With second derivative UV spectroscopic method, quantification of Ganciclovir was achieved by measurement of the peak-to through height of the signal corresponding to the second derivative of the direct spectrum at 253 nm. As indicated in Figure 4 (dissolution study), second derivative UV method gives highly comparable results to HPLC method. As expected, the accuracy of direct UV spectroscopic method suffers from substantial matrix interference.

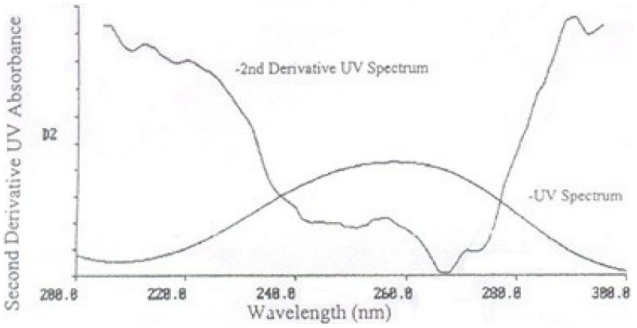

Figure 2 : UV and second derivative UV spectra of Placebo capsule solution

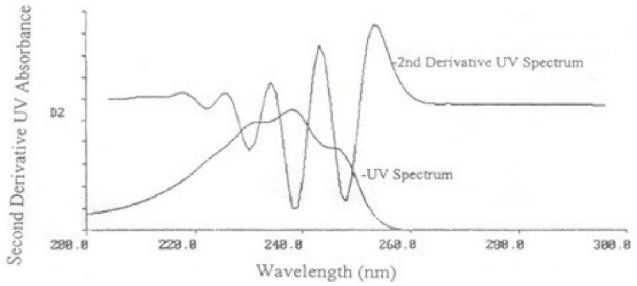

Figure 3 : UV and second derivative UV spectrum of Ganciclovir capsule

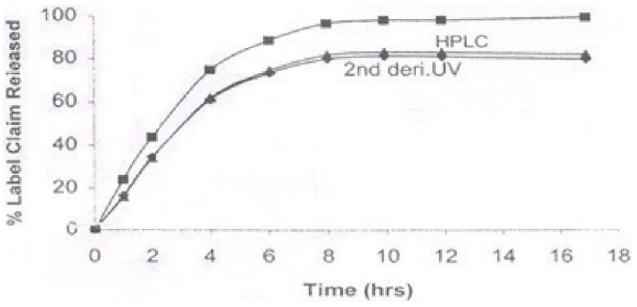

Figure 4 : Dissolution profile of Ganciclovir capsules obtained using 1. Direct UV, 2. Second derivative UV, 3. HPLC

METHOD VALIDATION

Linearity of second derivative spectra of Ganciclovir concentration was established by preparing one series of Ganciclovir solution ranging from 5 to 15 mg/ml which corresponds to 50–150% of method concentration (0.01 mg/ml). The second derivative spectra were recorded using the diluent as a blank. All solutions were measured for absorbency from 200 to 400 nm. Using regression analysis the following equation was obtained for Ganciclovir: y=0.048x+0.0014 (r^2=0.999) Where y is the absolute value of the second derivative of Ganciclovir absorbency at 253 nm and x is the concentration of Ganciclovir (mg/ml) Figure 5 (Linearity). The accuracy of the method was determined by investigating the recovery of the Ganciclovir at five levels ranging from 50 to 150% of the method concentration (0.01 mg/ml) from solution-spiked placebo. The results are shown in (TABLE 1), which indicate excellent recoveries ranging from 98.1 to 101.9% with a mean of 99.6% (RSD=1.0%, N=10). The measurement precision was determined by performing ten replicate injections of standard solutions at the method concentration (0.01 mg/ml). The RSD was found to be 1.0% by second derivative absorbency measurement (TABLE 2). The method precision for sample was determined by the analysis of ten Ganciclovir capsules. For quantification of Ganciclovir, the sample solutions were bracketed with external standard solutions. In addition, both HPLC and direct UV method analyzed the same

TABLE 1 : Accuracy of second derivative method determined by the recovery of Ganciclovir from placebo capsule spiked with Ganciclovir solution

Level (%)	mg Added	mg Recovered	% Recovery
50	10.74	10.83	100.3
50	9.99	10.12	101.5
75	15.66	15.84	100.9
75	15.35	15.28	99.4
100	20.74	20.71	99.8
100	20.36	20.11	99.0
125	24.90	24.60	99.0
125	25.32	24.94	98.5
150	29.86	29.33	98.1
150	30.31	30.05	99.1
Average			99.6
RSD (%)			1.0

TABLE 2 : Measurement precision

Injection	2nd UV reading	Injection	2nd UV reading
1	0.2121	6	0.2077
2	0.2082	7	0.2076
3	0.2074	8	0.2071
4	0.2068	9	0.2078
5	0.2081	10	0.2074
Average			0.2081
RSD (%)			1.0

TABLE 3 : Assay results for ten Ganciclovir developmental capsule solutions by UV, second derivative UV and HPLC methods

Sample (Capsule no.)	% Claim (UV)	% Claim (HPLC)	% Claim (2nd UV)
1	101.1	94.6	94.8
2	100.9	93.7	93.5
3	101.1	94.3	94.6
4	101.4	95.6	94.1
5	101.1	94.8	95.8
6	101.0	94.9	95.4
7	101.7	94.5	96.6
8	101.6	93.9	96.4
9	98.6	93.2	90.8
10	99.0	94.1	93.7
Average	100.7	94.4	94.6
RSD (%)	1.0	0.8	1.7

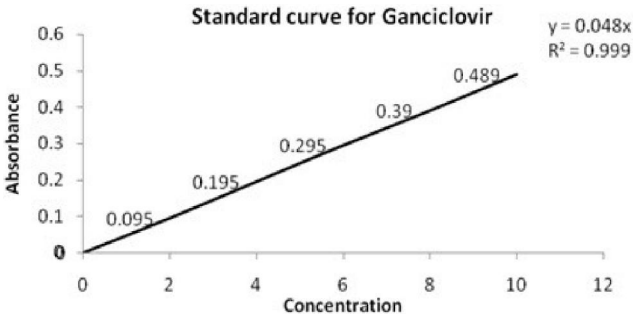

Figure 5 : Linearity of second derivative UV absorbance Vs Ganciclovir Concentrations 50-150% of ganciclovir method concentration (0.01mg/ml)

capsule solution. The results shown in TABLE 3 demonstrate that data generated by second derivative UV method agree well with HPLC results. In comparison with the data generated by second derivative UV, direct UV measurement has a standard error of 2–10%, further indicating background interference.

CONCLUSION

A reliable second derivative UV spectroscopic method was developed for the analysis of Ganciclovir in its developmental capsule dosage form. Two major features of this technique were observed during this study: (1) it is very efficient and offers high sample throughput by comparison with HPLC methods. Therefore, it undoubtfully renders in-time data turnaround during formulation development, and (2) it offers comparable accuracy by eliminating the interference of formulation excipients; unlike direct UV spectrometric method which often gave 2–10% standard error, resulted from matrix interference. Finally, this method can be used as an excellent alternative to HPLC for formulation screening, release and dissolution testing of Ganciclovir in the solid dosage form.

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