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Research Article

METHOD DEVELOPMENT AND VALIDATION OF BRIVUDINE BY UV SPECTROPHOTOMETRIC METHOD

Binu Varghese* and Sangeetha S

Department of Pharmaceutical Analysis, Prime College of Pharmacy, Prime Nagar, Near Govt. Polytechnic, Erattayal-678551, Palakkad, Kerala, India

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A B S T R A C T

A simple, accurate, rapid, precise and reproducible spectrophotometric method was developed and validated for the estimation of Brivudine in pharmaceutical dosage form. The developed UV spectrophotometric method for the estimation of Brivudine is based on the measurement of absorption at maximum wavelength 250 nm using methanol as the solvent. The stock solutions of Brivudine were prepared and subsequent suitable dilutions was prepared in methanol to obtain a standard curve. The standard solutions of Brivudine show absorption maxima at 250 nm. The drug obeyed Beer-Lambert's law in the concentration range of 2-10 µg/ml with regression 0.9997 at 250 nm. The overall % recovery do not differ significantly from 100 % which reflects that there was no interferences from common excipients used in tablet formulation. The low %RSD indicating accuracy and reproducibility of the method. The % RSD for inter-day and intra-day was found to be 0.3466 and 0.49 respectively which is <2% hence proved that the method is precise. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantitation as per ICH guidelines. The developed method can be adopted for the routine analysis of Brivudine in tablet dosage form as well bulk dosage form.

INTRODUCTION

Brivudine, 5-[(E)-2-bromoethenyl]-1-[(2R,4S,5R) -4-hydroxy-5- (hydroxymethyl) oxolan-2-yl] pyrimidine-2,4-dione (Figure 1) is an antiviral drug used in the treatment of herpus zoster ("shingles"). Like other antivirals, it acts by inhibiting replication of the target virus. These agents used in the prophylaxis or therapy of virus diseases. Some of the ways they may act include preventing viral replication by inhibiting viral DNA polymerase; binding to specific cell-surface receptors and inhibiting viral penetration or uncoating; inhibiting viral protein synthesis; or blocking late stages of virus assembly. Brivudine is an analogue of the nucleoside thymidine. The active compound is Brivudine 5'-triphosphate, which is formed in subsequent phosphorylations by viral (but not human) thymidine kinase and presumably by nucleoside-diphosphate kinase. Brivudine 5'-triphosphate works because it is incorporated into the viral DNA, but then blocks the action of DNA Polymerase, thus inhibiting viral replication[1,2]. it is a white powder, soluble in water and methanol. As per investigation of literature, various analytical method were developed for analysis of Brivudine[3,4]. The aim of this work to develop a simple, accurate, rapid, precise and reproducible spectrophotometric method for quantitative determination of Brivudine[7,10,11]. In this method we developed a method for determination of Brivudine in bulk drug sample and the tablet dosage form and validated.

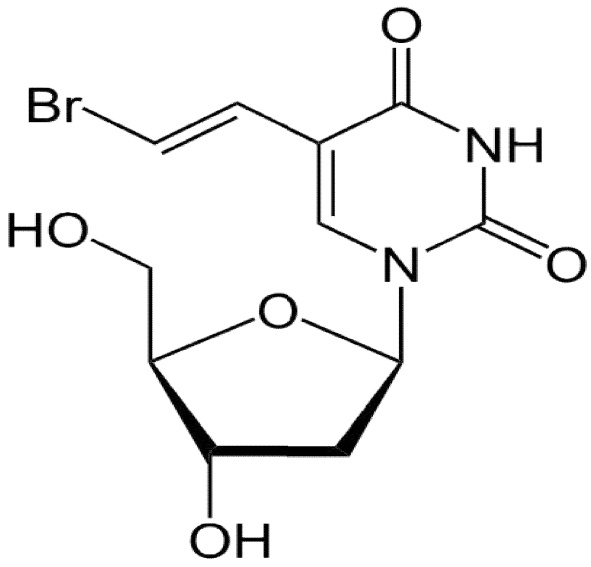

Figure 1 Brivudine

Corresponding author:* **Binu Varghese
Department of Pharmaceutical Analysis, Prime College of Pharmacy, Prime Nagar, Near Govt. Polytechnic, Erattayal-678551, Palakkad, Kerala, India

MATERIALS AND METHOD

Instrument

Shimadzu UV 1800 double beam spectrophotometer with UV probe software version 2 to develop the analytical method. The above instruments had automatic wavelength accuracy 0.1 nm and matched quartz cell with 1 cm path length and weighing balance (Shimadzu) were used for this work.

MATERIAL

Brivudine was gifted from Apex Company. The commercially available tablet Zostex 125 mg was obtained from the market. Methanol (Laboratory grade) was used as a solvent was obtained from s-d fine-chem limited, Mumbai.

METHOD DEVELOPMENT

Preparation of standard Solution

10 mg of pure drug (Brivudine) was weighed transferred and made up to 10 ml with methanol in a standard flask previously rinsed with methanol to get concentration of 1000 µg/ml (Standard stock I). From the stock 1ml was diluted to 10 ml to get the concentration of 100 µg/ml (Standard stock II).
Selection of wavelength

Accurately measured 0.2 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted to 10 ml with methanol to give a concentration of 2 µg/ml of Brivudine was prepared and scanned in the range of 200-400nm against methanol as a blank, to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution.

Preparation of Working Standard Solution

From the stock solution, containing 100 µg/ml of Brivudine about 0.2-1ml were transferred to 10 ml of volumetric flask to get concentration of about 2-10 µg/ml.

The absorbances were noted for all the standard solutions of Brivudine at selected wavelength. Standards were scanned in the wavelength range of 200-400 nm. The linear graph was plotted between concentrations versus absorbance.

METHOD VALIDATION

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness.

Linearity and Range

The linearity of an analytical method is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an anlyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis at a concentration range of of 2-10 µg/ml. the absorbance obtained at respective concentration was recorded and the calibration graph is plotted as concentration (µg/ml) verses absorbance. The linear regression equation and coefficient correlation were obtained from the UV probe software.

Precision

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.
Precision of method was demonstrated by

1. Intraday precision
2. Interday precision

Intra Day Precision

Intraday precision was studied by three different concentrations in the linearity range of the drug for three times on the same day and the absorbances were recorded. All the study was performed in triplicates and %RSD was calculated.
Inter Day Precision

Interday precision was studied by standard drug at three concentrations in the linearity range of the drug for three days over a period of one week and the absorbances were recorded. All the study was performed in triplicates and % RSD was calculated.

Lod&Loq

The detection limit of an individual analytical method is the lowest amount of analyte in a sample, which can be detected, but not necessarily qunatitated as an exact value.

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantities.

From the limit of detection and limit of quantification the sensitivity of the method was determined. LOD & LOQ was determined by preparing solutions of different concentrations from 2-10 µg/ml. The LOD and LOQ was calculated using an equation

$LOD=3.3\sigma/S$
$LOQ=10\sigma/S$
Where,
σ=Standard Deviation
S =Slope

Robustness

The robustness of an analytical method is a measure of its capacity remains unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method was studied using 2 µg/ml of the Brivudine solution and analysed by a change in wavelength. The wavelength was selected λ_{max} ±1 i.e. 249 and 251 nm respectively for the standard Brivudine solution.

Stability Studies (Ruggedness)

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analysts, different instruments and different days. When the prepared solutions are exposed to atmosphere, the analytes are likely to decompose. Hence it is a necessary to conduct stability studies. Stability of the analytes in the solutions was studied using the solutions of 2 µg/ml of standard Brivudine solution and the absorbance was compared with the absorbance of freashly prepared solutions with the change in different days.

Recovery Studies

The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or accepted reference value and the value found. This is sometimes termed as trueness. The accuracy of the proposed method was determined based on the recovery study. To study the accuracy of the proposed method, standard addition procedure was adopted. To a pre-analyzed sample solution a known quantity of standard Brivudine was added at 100 % and 120 % levels. %Recovery was calculated from the ratio of difference between the amount of drug found after the addition of standard drug and amount of analyte present in pre-analyzed formulation to that of the standard Brivudine added to the formulation. %RSD was calculated.

Application of The Preposed Methods For Analysis of Formulation 20 tablet each containing 125 mg of Brivudine were taken and average weight was calculated. They were finely pulverized and the quantity of homogenized powder equivalent to 10mg of Brivudine was transferred to 10 ml volumetric flask and made up to the volume with methanol. Then the solution was filtered using whatmann filter paper and observed by UV analtsis the procedure was repeated triplicate. The content of Brivudine was calculated. %RSD was calculated.

RESULTS AND DISCUSSION

Selection of solvent

Solubility of the drug was checked in solvents like water, methanol, ethanol and acetonitrile. UV spectra of the drug in those solutions were recorded. Absorbance of the drug was higher and exhibited distinct λ_{max} in methanol and hence methanol was selected as the solvent for the further studies.

Selection of wavelength

From the stock solution of 1000 µg/ml of Brivudine, a solution containing 2 µg/ml of Brivudine was prepared and scanned in the UV range of 200-400 nm against methanol as blank. Brivudine shows good absorption spectrum at 250 nm, where linear response was very good with maximum acceptable absorbance (Figure 2). Hence the wavelength was selected for the studies.

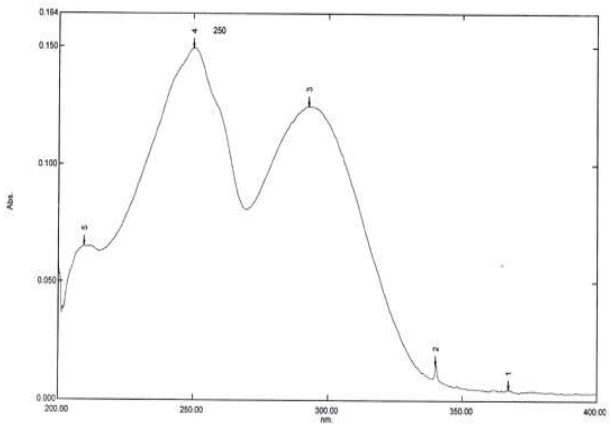

Figure 2 Spectrum of Brivudine

Validation of the Method

The developed method was validated in terms of parameters like linearity and range, precision, LOD & LOQ, stability studies and recovery.

Linearity and Range

Linear regression data showed good correlation coefficient over a percentage range of 2-10 µg/ml. The absorbance of these solutions was noted at the selected wavelength 250 nm (Table 1). The calibration curve was plotted using the concentration and absorbance (Figure 3). The slope, intercept and correlation coefficient values were noted (Table 2).

Table 1 Calibration data of Brivudine

Concentration (µg/ml)	Absorbance at 250 nm
2	0.104
4	0.137
6	0.174
8	0.208
10	0.244

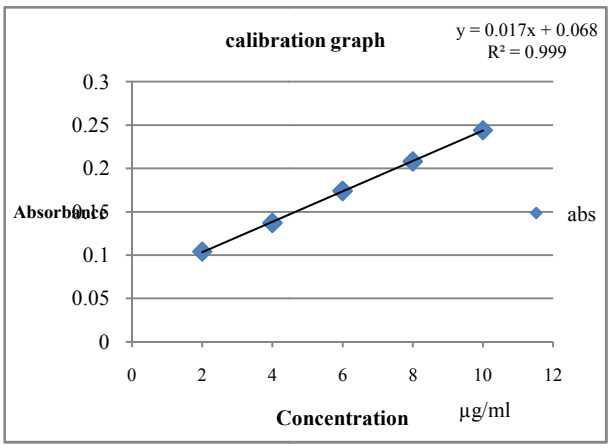

Figure 3 Calibration curve of Brivudine

Table 2 Regression data of Brivudine

Linear regression data	Brivudine
Slope	0.017
Intercept	0.068
Correlation coefficient	0.999
Linearity range	2-10 µg/ml

Precision

Precision of the method was demonstrated by

- Intraday precision
- Interday precision

Intraday precision

Intraday precision was found out by carrying out the analysis of the standard drug for three different concentrations in the linearity range of the drug for three times on the same day and % RSD was calculated (Table 3).

Table 3 Intraday precision

Brivudine				
Concentration (µg/ml)	Absorbance at 250 nm	Mean	SD	%RSD*
2	0.1041 0.1050 0.1041	0.1044	0.00058	0.55
6	0.1750 0.1731 0.1740	0.1740	0.0009	0.54
10	0.2450 0.2440 0.2431	0.2440	0.0009	0.38

*mean of three observations

Interday precision

Interday precision was found out by carrying out the analysis of the standard drug for three different concentrations in the linearity range of the drug for three days over a period of one week and % RSD was calculated (Table 4).

Table 4 Interday precision

Day	Concentration (µg/ml)	Brivudine Absorbance at 250 nm	Mean	SD	%RSD*
1	2	0.1040	0.1043	0.005	0.50
2		0.1051			
3		0.1040			
1	6	0.1740	0.1736	0.006	0.37
2		0.1741			
3		0.1728			
1	10	0.2441	0.2447	0.0004	0.17
2		0.2450			
3		0.2450			

*mean of three observations

lod & loq

Brivudine in the developed method was estimated. LOD & LOQ of the formulation was considered acceptable (Table 5).

Table 5 LOD & LOQ Study

	Brivudine
LOD	1 µg/ml
LOQ	2 µg/ml

Robustness

Robustness of the method was determined by analysing the standard Brivudine solution of 2 µg/ml at different wavelength (λ_{max} ±1). Absorbance was measured. Results of robustness indicate that the selected factor remained unaffected by small variation which confirms the robustness of the method (Table 6).

Table 6 Robustness data

Wavelength (nm)	Absorbance	Mean	SD	%RSD
249	0.1041	0.1037	0.0005	0.48
249	0.1030			
249	0.1041			
250	0.1041	0.1044	0.00058	0.55
250	0.1050			
250	0.1041			
251	0.1041	0.1037	0.0005	0.48
251	0.1030			
251	0.1041			

Stability Studies (Ruggedness)

In the ruggedness or stability study, the influence of small deliberate variations of the analytical parameters on the absorbance of the drug was examined. When the prepared solutions are exposed to atmosphere, the analytes are likely to decompose. Hence it is a necessary to conduct stability studies. Stability of the analytes in the solutions was studied and the absorbance was compared with the absorbance of freshly prepared solutions. The solutions where found to be stable for about 48hrs and 3 days in refrigeration as the reduction of absorbance was within limits (Table 7).

Table 7 Stability studies

Day	Brivudine Concentration (µg/ml)	Absorbance at 250 nm	Mean	SD	% RSD*
Day 1	2	0.1041	0.1044	0.0058	0.55
		0.1050			
		0.1041			
Day 2		0.1041	0.1037	0.0004	0.45
		0.1031			
		0.1041			
Day 3		0.1041	0.1044	0.0004	0.45
		0.1051			
		0.1041			

*mean of three observations

Recovery Studies

In order to ensure the suitability and reliability of the proposed method recovery studies were carried out. To a pre analysed sample solution a known quantity of standard Brivudine was added and the recovery studies were carried out at 100% and 120% levels. The % recovery and % RSD of the results were calculated (Table 8). The value proves the accuracy of the method. %Recovery was calculated using the formula:

$$\left[\begin{array}{l}\text{Amount of drug found after}\\ \text{the addition of standard drug}\end{array} - \begin{array}{l}\text{Amount of drug found}\\ \text{before}\\ \text{the addition of standard}\\ \text{drug}\end{array}\right] \times 100$$

Amount of standard drug added

%RSD was calculated using the formula
Standard deviation / mean × 100

Table 8 Recovery study data

	%Recovery	%RSD*
100%	99.87	0.8
120%	99.62	0.6

*mean of six observations

Analysis of Formulation

20 tablets each containing 125 mg of Brivudine were taken for the study and average weight was determined quantity equivalent to 10 mg of Brivudine was weighed and transfered to a volumetric flask and make up with methanol. It was filtered and used for analysis. The amount of drug was calculated (Table 9).

Table 9 Analysis of formulation

Drug	Amount of drug/tablet (mg)		%Labelled claim	%RSD*
	Labelled	Estimated		
Zostex	125	124.2	99.6%	0.6

*mean of six observations

CONCLUSION

A simple, rapid and precise spectrophotometric method has been developed for the quantitative estimation of Brivudine in bulk and pharmaceutical formulation. The method was validated as per ICH guidelines, and it is found that the developed method is robust and specific. Hence, the method can be successfully and suitably acquired for routine quality control and analysis of Brivudine in bulk and pharmaceutical dosage form.

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