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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TEMOZOLOMIDE IN PHOSPHATE BUFFER PH 2.0 AS A SOLVENT BY UV SPECTROSCOPY

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ABSTRACT
Temozolomide is an antineoplastic agent with activity against a broad spectrum of murine tumors. This compound is currently marketed for the treatment of patients with glioblastoma multiforme and anaplastic astrocytoma, which are serious and aggressive types of brain cancers. The present research work discusses the development and validation of a UV spectrophotometric method for temozolomide. Simple, accurate, precise and cost efficient spectrophotometric method has been developed for the estimation of temozolomide in bulk and capsule dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 330 nm in Phosphate Buffer pH 2.0. The percentage recovery of temozolomide was found to be in range 98.47 – 100.4 %. Beers law was obeyed in the concentration range of 4-18 µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation $y = 0.053x + 0.006$ with r^2 of 0.999 was obtained. Validation was performed as ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The proposed method may be suitable for the analysis of temozolomide in bulk and capsule formulation for quality control purposes.
Keywords: Temozolomide, UV spectrophotometer, anaplastic astrocytoma, ICH guidelines.

INTRODUCTION

Temozolomide is an oral alkylating agent which can be used for the treatment of Grade IV astrocytoma - an aggressive brain tumor, also known as glioblastoma multiforme as well as melanoma, a form of skin cancer. It is also indicated for relapsed Grade III Anaplastic Astrocytoma and not indicated for, but now used to treat oligodendroglioma brain tumors in some countries, replacing the older (and less well-tolerated) PCV (Procarbazine-Lomustine-Vincristine) regimen. The agent was developed by Malcolm Stevens[1]. A derivative of imidazotetrazine, temozolomide is the prodrug of MTIC (3-methyl-(triazen-1-yl) imidazole-4-carboxamide) (Figure 1). Review of Literature for Temozolomide analysis revealed that several existing methods including different technique such as HPLC[2-4], LC/MS/MS[5-7], Capillary Electrophoresis[8], Capillary Chromatography[9] assay have been reported for assay of Temozolomide. However there is no simple and accurate method reported for the detection of Temozolomide in pharmaceutical formulation by UV spectrophotometric. The aim of present work is to develop a simple, sensitive, specific, cost effective spectrophotometric method for the determination of Temozolomide in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments

Electronic Weighing balance - single (pan balance, Model Axis LC/GC), Digital pH meter (Model- Systronics), Sonicator- Ultra Sonicator (Model- Bandelin sonorex), Double Beam UV-Visible spectrophotometer - Schimadzu 1800. UV spectra of standard and sample solutions were recorded in 1 cm quartz cells at the wavelength ranges of 200-400 nm.

Chemicals and Reagents

Temozolomide was obtained as a gift sample from Natco Pharma, Ltd, Hyderabad, India. Methanol A.R, potassium dihydrogen phosphate A.R were purchased from Merck, Hydrochloric acid, Sodium hydroxides were purchased from SD Fine Chem, Mumbai, India.

Experimental Methods

Preparation of Standard Solution

Standard Temozolomide (100 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved properly and diluted up to the mark with Phosphate buffer pH 2.0 to obtain concentration of 1 mg/ml. This solution was used as working standard solution. From this solution, by suitably dilution, 10 µg/ml concentrations was prepared and used as working standard solution.

Preparation of sample solution

Weigh accurately about powder equivalent to 100 mg of temozolomide capsule contents in to 100 ml volumetric flask and dissolved in 50 ml of Phosphate buffer pH 2.0 and mixed well, then volume was made upto the mark with the same. Final dilution of 10 µg/ml was prepared from above solution. The solution was scanned in UV region (200 nm – 400 nm).

RESULT AND DISCUSSION

Analytical method development

To develop accurate, precise and sensitive UV spectrophotometric method for Temozolomide various solvent systems such as water, methanol, ethanol, Phosphate buffer pH 2.0 and 0.1 N HCl etc were tried alone and in combinations. Selection of Phosphate buffer pH 2.0 was based on sensitivity, minimal interference, ease of preparation, suitability for drug content estimation, stability, analysis time and cost. The λ max for Temozolomide in Phosphate buffer pH 2.0 was found to be 330 nm (Figure 2) the method showed linear relationship (with correlation coefficient of 0.999) in the concentration range of 4-18 µg/ml (Figure 3).

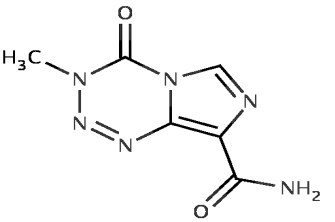

Figure 1: Chemical structures of Temozolomide

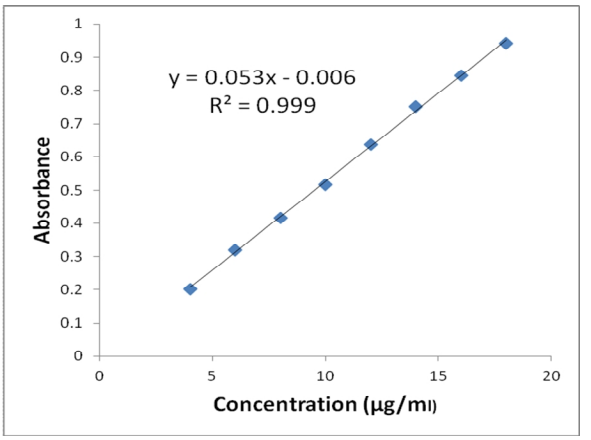

Figure 2: Calibration curve for Temozolomide in phosphate buffer pH 2.0

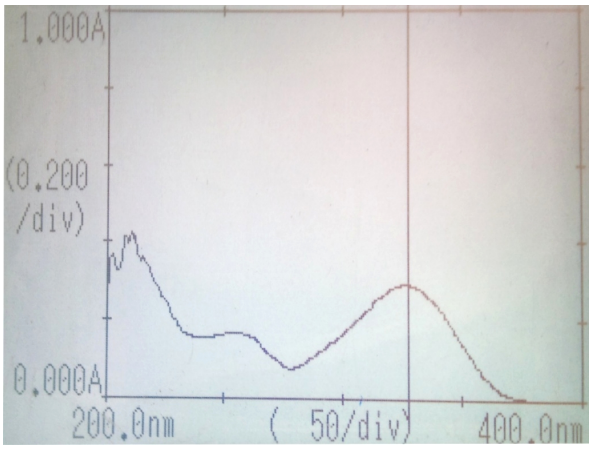

Figure 3: Phosphate buffer pH 2.0 λ max 330 nm

Table 1: Data of Calibration Curve for Temozolomide in Phosphate Buffer

Concentration (µg/ml)	Absorbance
4	0.201
6	0.32
8	0.416
10	0.516
12	0.638
14	0.752
16	0.846
18	0.942

Table 2: Intraday Precision Data for Temozolomide

Parameter	Temozolomide λ max at 330 nm	
	Standard	sample
Absorbance	0.514	0.465
	0.516	0.464
	0.519	0.468
Mean	0.516333	0.465667
SD	0.002517	0.002082
% RSD	0.487401	0.447029

Table 3: Interday Precision Data for Temozolomide

Parameter	Day-1		Day-2	
	Std λ max in phosphate buffer pH 2	Sample λ max in phosphate buffer pH 2	Std λ max in phosphate buffer pH 2	Sample λ max in phosphate buffer pH 2
Day to day	0.520	0.486	0.515	0.484
	0.522	0.486	0.513	0.481
	0.520	0.485	0.517	0.481
Mean	0.5206	0.4856	0.5150	0.482
SD	0.0011	0.005	0.002	0.0017
% RSD	0.2218	0.1188	0.388	0.3593

Table 4: Results of Recovery

Parameter / Drug conc.	Absorbance in phosphate buffer	Amount present (µg/ml)	%Recovery (Average of three replicates)	Mean % Recovery
80 %	0.486	8	98.47	99.58
	0.485			
	0.487			
100 %	0.493	10	99.88	
	0.492			
	0.496			
120 %	0.501	12	100.4	
	0.498			
	0.494			

Table 5: Summary of Validation Parameters of Temozolomide

Parameters	Results
Beers law limit (µg/ml)	1-19
Molar absorptivity (L mol^{-1} $cm^{-1)}$	0.0516
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.0516
Correlation coefficient (r^2)	0.999
Regression equation (y = mx+c)	y = 0.053 x - 0.006
Slope (m)	0.053
Intercept (c)	0.006
LOD (µg/ml)	0.1058
LOQ (µg/ml)	0.3207
Standard Error	0.0007

Analytical method validation

The developed method was validated according to ICH guidelines[10,11]

Linearity and Range

Various concentrations were prepared from the secondary stock solution (500 µg/ml) ranging from 4-18 µg/ml. The samples were scanned in UV-VIS Spectrophotometer against Phosphate buffer pH 2.0 as blank. The calibration curve of Temozolomide (Figure 3) was plotted between concentration of Temozolomide and respective measured absorbance values at 330 nm. It was found to be linear in the specified range and the regression coefficient was found to be 0.999. The results were shown in Table 1.

Precision

The precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time in the two consecutive days. The % RSD value of intraday and inter-day analysis was found to be 0.488 and 0.222 for Temozolomide (10 µg/ml) in Phosphate buffer pH 2.0. The results were shown in Table 2 and 3.

Accuracy (Recovery)

The accuracy of the method was evaluated by recovery studies. A known quantity of Temozolomide was added at different levels (80,100 and 120 %). The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98.47–100.4 % for Temozolomide in Phosphate buffer pH 2.0. The recovery data was shown in Table 4.

Assay of marketed formulation

The proposed method was applied to analyze commercially available Temozolomide capsules having content equivalent to 20 mg. Ten capsules were weighed and powder equivalent to 100 mg transferred in 100 ml volumetric flask and dissolved in Phosphate buffer pH 2.0 finally volume was made up to mark with the same. The solution was then filtered through Wattman filter paper #41. This filtrate was diluted suitably with solvent to get the solution of 10 µg/ml. The absorbance was measured against Phosphate buffer pH 2.0 as blank. The readings were taken in triplicate by performing the same experimentation in three times. The % Purity and content of the drug in capsule dosage form was calculated. The mean assays of six replicate samples were found to be 100 %.

$$\%\text{purity} = \frac{\text{Test absorbance x std dilution x avg wt x 100}}{\text{Std absorbance x test dilution x labeled claim}}$$

DISCUSSION

Solubility studies were performed in different solvents such as water, methanol, 0.1N HCl, 0.1N NaOH and Phosphate buffer pH 2.0. The drug was found to be freely soluble in Phosphate buffer pH 2.0 and shown considerable absorbance values at 330 nm. Phosphate buffer pH 2.0 was taken as blank for further work. Form the stock solution (1 mg/ml) different concentration as of solutions like 4-18 µg/ml were prepared and absorbance was measured at 330 nm. Calibration curve was prepared by plotting graph between absorbance vs concentration (µg/ml) (Figure 1). The data was statistically validated by means of least square regression method. The detection and quantization limits were found to be 0.1058 µg/ml and 0.3207 µg/ml respectively. The precision (intraday and interday) results showed good reproducibility with % RSD below 2. This indicates that method was precise. The accuracy of the method was performed by recovery studies at 8 µg/ml, 10 µg/ml and 12 µg/ml. the percentage recovery was found to be 98.47, 99.88 and 100.4 % respectively. This indicates that the method was accurate. The proposed method was applied for the assay of Temozolomide capsules and the results were tabulated in Table 5.

CONCLUSION

The developed UV spectrophotometric method for the estimation of Temozolomide was found to be simple and useful with high accuracy, precision, and reproducible. Sample recoveries in all formulations using the above method were in good agreement with their respective label claim or theoretical drug content, this suggesting the validity of the method and non interference of formulation excipients in the estimation. The developed method was applied for routine quality control analysis of Temozolomide capsules.

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