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Original Article

SPECTROSCOPIC DETERMINATION OF TEMOZOLAMIDE IN BULK AND ITS CAPSULE DOSAGE FORM

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ABSTRACT

Objective: To develop two simple, precise, accurate and sensitive visible spectrophotometric methods for the quantitative estimation of Temozolamide (TMZ) in bulk drug as well as in pharmaceutical dosage forms (capsules).

Methods: Method A is based on the complex formation reaction of TMZ with Bromothymol blue in presence of chloroformto form Golden Yellow colored chromogen with absorption maximum at 430 nm. Method B is based on the complex formation reaction of TMZ with Mordant Black in presence of chloroform to form Blood red colored chromogen with absorption maximum at 515 nm.

Results: Beer's law is obeyed in the concentration range of 10-60 µg/mL. LOD and LOQ of the Method A was found to be 0.223 µg/mL and 0.561 µg/mL respectively. Beer's law is obeyed in the concentration range of 8 - 20 µg/mL. LOD and LOQ of the Method B was found to be 0.143µg/mL and 0.484 µg/mL respectively.

Conclusion: The proposed methods were statistically validated as per ICH guidelines and found to be useful for the routine determination of TMZ in capsules.

Keywords: Visible spectrophotometric methods, Temozolamide, Bromothymol blue, Mordant Black.

INTRODUCTION

Temozolomide (TMZ) medication is used to treat certain types of brain cancer. The chemical structure of TMZ was shown in Fig. 1. It is a chemotherapy drug that works by slowing cancer cell growth. In some patients, TMZ decreases the size of brain tumors. The therapeutic benefit of TMZ depends on its ability to alkylate/methylate DNA, which most often occurs at the N-7 or O-6 positions of guanine residues. This methylation damages the DNA and triggers the death of tumor cells. However, some tumor cells are able to repair this type of DNA damage, and therefore diminish the therapeutic efficacy of temozolomide, by expressing an enzyme called O-6- methylguanine-DNA methyl transferase (MGMT) or O-6-alkylguanine-DNA alkyl transferase (AGT or AGAT) [1-4].

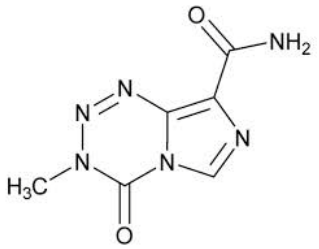

Fig. 1: Chemical structure of Temozolomide

Review of Literature for Temozolomide analysis revealed that several existing methods including different technique such as HPLC [5,6,7] LC/MS/MS [8,9,10] Capillary Electrophoresis [11], Capillary Chromatography [12], UV spectroscopic methods [13, 14] in different solvents, have been reported for assay of TMZ. However there is no simple and accurate method reported for the detection of TMZ in pharmaceutical formulation by visible spectrophotometry. The aim of present work is to develop a simple, sensitive, specific, cost effective visible spectrophotometric method for the determination of Temozolomide in bulk and pharmaceutical dosage form (capsules).

MATERIALS AND METHODS

Instruments

Electronic Weighing balance - single (pan balance, Model Axis LC/GC), Digital pH meter (Model- Systronics), Sonicator- Ultra Sonicator (Model- Bandelinsonorex), Double Beam UV-Visible spectrophotometer - Schimadzu 1800. UV spectra of standard and sample solutions were recorded in 1cm 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. Methanol A.R, potassium dihydrogen phosphate A.R were purchased from Merck. Bromothymol blue and Mordant Black was purchased from SD Fine chem, Mumbai. Distilled water was prepared in house. All other chemicals used were AR grade.

Preparations of Buffer and Reagents

Preparation of Acidic Phosphate Buffer pH 2.5

100 gm of Potassium dihydrogen phosphate was dissolved in 800 ml of distilled water with stirring. The pH was adjusted to 2.5 with 0.1 N HCl. The final volume was made upto 1000 ml.

Preparation of Bromo Thymol Blue

70 mg of Bromo thymol blue and 0.72 ml of 0.1 M NaOH was dissolved in 20 ml of Ethanol. The solution was stirred for the complete dissolution and the volume was made upto 1000 ml with distilled water.

Preparation of Mordant Black

0.2 g of Erichrome black and 2gms of hydroxylamine HCL was dissolved in methanol with stirring, and the final volume was made upto 50 ml with the same.

Preparation of standard stock solution

A standard stock solution of TMZ was prepared by dissolving 100 mg of TMZ in distilled water in a 100 ml volumetric flask, the final

volume was made upto the mark with the same to get the final concentration of 1mg/ml.

Preparation of working standard solution

A working standard solution containing 100µg/ml was prepared by diluting the above stock solution. The fresh working standards were prepared daily.

Assay of temozolomide

Method A: With Bromothymol blue

To a 25ml seperating funnel, 1ml of working standard drug solution, 2ml of acedic phosphate buffer pH 2.5, 5ml of Chloroform were added and stirred well for few minitues. 2ml of Bromo thymol blue reagent was added and mixed vigorously for 2 minutes. The mixture was kept a side for few minutes to separate the aqueous and orgainc layers. A Golden yellow colour complex orgainc layer was seperated which was used for further analysis. A colourless blank was prepared with similar manner without drug solution.

Method B: With Mordant Black

To a 25ml seperating funnel, 1ml of working standard drug solution, 2ml of acedic phosphate buffer pH 2.5, 5ml of Chloroform were added and stirred well for few minitues. 2ml of Mordant Black reagent was added and mixed vigorously for 2 minutes. The mixture was kept a side for few minutes to separate the aqueous and orgainc layers. A Blood red color complex orgainc layer was seperated which was used for further analysis. A colourless blank was prepared with similar manner without drug solution.

Preparation of sample solution (for Assay of TMZ capsules)

20 capsules of TMZ were accurately weighed and powered. Capsule powder equivalent to 100 mg of TMZ was dissolved in 5.0 mL of distilled and made up to 100 mL with distilled water, sonicated for 15 min and filtered. The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample. The analysis procedure was repeated three times with capsule formulation and the results of analysis were shown in Table 1.

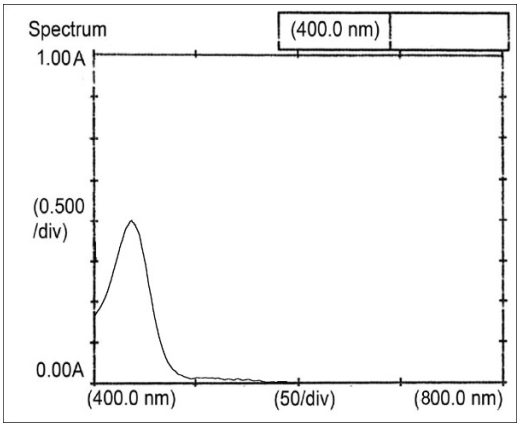

Fig. 2: λmax of Temozolamide 430 nm (Method 1)

RESULTS AND DISCUSSION

Method A

Bromo thymol blue has been used as a color-developing reagent in the spectrophotometric determination of pharmaceutical drug compounds [15-16]. The reaction of TMZ with bromothymol blue to produce a golden yellow charge transfer product of the n-π* type, this compound is considered to be an intermediate molecular association complex which dissociates in the corresponding radical anion in the solvent.

At optimum conditions the radical anion (absorbing species) in the medium after mixing of the reagent and showed maximum absorption at 430 nm. Fig. 2 shows the λmax curve. The absorbance was found to increase linearly with increasing concentration of TMZ. The effect of pH of buffer was studied by forming the colored product in the presence of various buffers pH the absorbance of the proton transfer product was measured. Phosphate buffer pH 2.5 was optimised. The reaction time was determined by following the color development at room temperature.

Method B

TMZ exhibits maximum absorbance (λmax) at 515 nm. TMZ was found to react with Mordent black under the experimental conditions to form an blood red coloured product exhibiting λmax at 515 nm. The λmax curve was shown in Fig. 3. Under the optimum reaction conditions, the absorbance was found to obey the Beer–Lambert law. The effects of pH on the reaction of TMZ with mordent black were examined by varying the pH from 1.0 to 5.0, the results revealed that the absorbance increased with increasing up to pH 3. The maximum readings were attained at pH value of 2.5. At pH value more than 3, a decrease in the reading occurred. This was attributed probably to the increase in the amount of hydrogen ion that holds back the reaction of TMZ with mordent black.The effect of temperature on the reaction was also studied by varying the temperature from 25°C to 40°C. The highest absorbance is obtained at room temperature for 10 min.

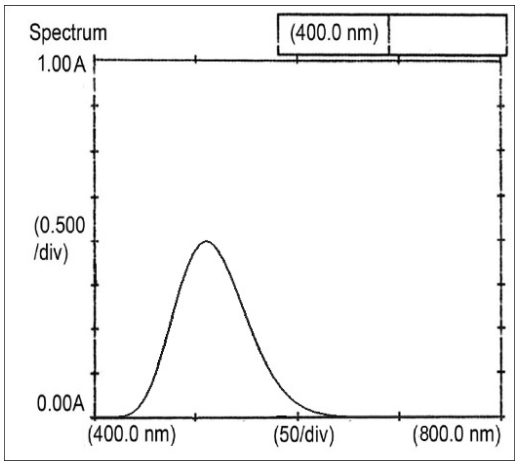

Fig. 3: λmax of Temozolamide 515 nm (Method 2)

Validation of the proposed method

Linearity and sensitivity: Calibration curves for Methods A and B in the ranges 10-60 µg/mL and 8-20 µg/mL were linear with correlation coefficients (r2) of 0.9983 and 0.9981

for methods A and B, respectively. The molar absorptivities (ε) at 430 nm and 515 nm for Methods A and B were 1.86 x 10^{-8} and 2.05 x 10^{-8} L/mole/cm, respectively. The sandell's sensitivity values were 0.0831 and 0.0189 for methods A and B respectively. The calibration curves of method A and B were shown in Fig. 4 and 5 respectively.

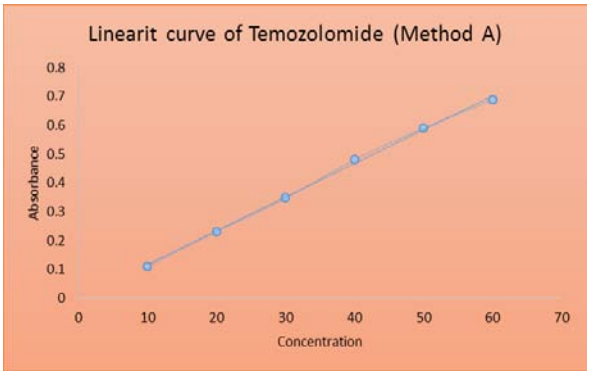

Fig. 4: Linearity curve of Temozolomide (Method A)

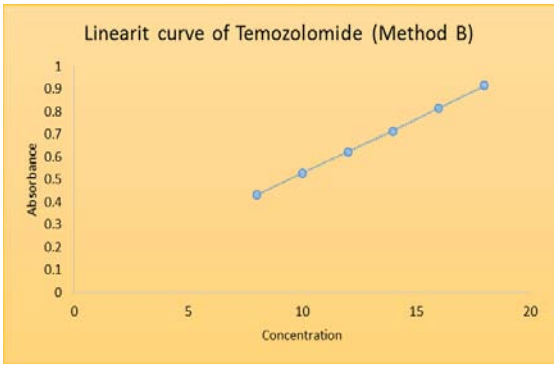

Fig. 5: Linearity curve of Temozolomide (Method B)

LOD and LOQ

The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected for the two methods was calculated using the following equation

LOD= 3.3 X δ/ s

In accordance with the formula, the detection limits were found to be 0.223 and 0.143 µg/mL for method A and B, respectively.

The limit of quantification (LOQ) is defined as the lowest concentration that can be measured with acceptable accuracy and precision.

LOQ=10 X δ / s

Where

δ = Standard deviation

s = slope of calibration curve

According to this equation, the limit of quantification was found to be 0.561 and 0.484 µg/mL for Method A and B respectively; these parameters for the two methods were summarized in Table 1.

Accuracy and precision

The accuracy and precision of the proposed method were determined at three concentration levels of TMZ (within the linear range) by analyzing three replicate analyses on pure drug of each concentration. The percentage relative error as accuracy and percentagerelative standard deviations (RSD) as precision for the results did not exceed 2% for the two methods as shown in Table 1, indicating the good reproducibility and repeatability of the two methods. This good level of precision and accuracy was suitable for quality control analysis of TMZ in their pharmaceutical formulation.

Applications of the methods

The proposed methods were applied to the pharmaceutical formulation containing TMZ. The results are shown in Table 1. Indicate the high accuracy of the proposed methods for the determination of the studied drug. The proposed methods have the advantage of being virtually free from interferences by excipients. The percentages were 99.06 ± 0.46 and 99.75 ± 1.67 for method A and B, respectively (Table 1).

Table 1: Summary of Validation results of Method A and B

Parameters	Results	
	Method A	**Method B**
Lamda max (ƛmax)*	430 nm	515 nm
Beer's law limits (µg/ml)	10-60	8-20
Molar absorptivity(L/mol/cm)	1.86 x 10^{-8}	2.05 x 10^{-8}
Sandell's sensitivity (µg/cm2/0.001absorbance unit)	0.0831	0.0189
Regression equation	Y=0.0117x-0.0007	Y = 0.0496x+0.0194
Slope(m)	0.0117	0.0496
Intercept(c)	-0.0007	0.0194
Correlation coefficient (r2)	0.9983	0.9981
Precision (%RSD)*	0.8	0.54
LOD	0.223	0.143
LOQ	0.561	0.484
Assay (% Purity)**	99.06	99.75

* Average of 6 replicate samples.

CONCLUSION

The development spectro photometric methods for the determination of TMZ in pharmaceutical formulation were simple, sensitive, rapid and accurate. The methods are practical and valuable for routine application in quality control laboratories for analysis of TMZ.

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