



International Journal of Innovative Research in Science Engineering and Technology (IJIRSET)

(A Monthly, Peer Reviewed, Refereed, Scholarly Indexed, Open Access Journal)



Impact Factor: 8.699

Volume 15, Issue 3, March 2026

A Simple and Sensitive Spectrophotometric Method for the Quantification of Temozolomide Using MBTH Reagent

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ABSTRACT

Objective:

The objective of this study was to develop a simple, accurate, sensitive, and reliable spectrophotometric method for the quantitative determination of temozolomide in pharmaceutical dosage forms and biological samples.

Methods:

The proposed method is based on the formation of a green-colored chromogen resulting from the oxidative coupling reaction between temozolomide and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of chloroform. The absorbance of the resulting chromogen was measured at 620 nm.

Results:

Beer's law was obeyed over the concentration ranges of 5–30 µg/mL for pharmaceutical formulations and 4–24 µg/mL for blood samples. The limits of detection (LOD) and quantification (LOQ) were found to be 0.257 µg/mL and 0.784 µg/mL for formulations, and 0.1726 µg/mL and 0.5180 µg/mL for blood samples, respectively. The percentage relative standard deviation (%RSD) values were 1.960 for formulations and 0.05901 for blood samples, indicating good precision. The percentage recoveries were found to be 99.76% for pharmaceutical formulations and 99.94% for blood samples, demonstrating excellent accuracy.

Conclusion:

The proposed method was successfully validated in accordance with ICH guidelines and was found to be simple, precise, accurate, and reproducible. The method is suitable for routine quantitative analysis of temozolomide in bulk drug and pharmaceutical dosage forms.

KEYWORDS: UV–Visible spectrophotometric method; Temozolomide; Blood sample; MBTH; ICH guidelines.

I. INTRODUCTION

Temozolomide (TMZ) is a novel alkylating agent widely used in anticancer chemotherapy, with a molecular formula of C₆H₆N₆O₂ (Fig. 1). It was initially synthesized in the United Kingdom and later developed by Schering-Plough (USA). Temozolomide received regulatory approval in Europe in 1998 and subsequently in the United States in 1999 under the trade name Temodal. It is primarily indicated for the treatment of glioblastoma multiforme and has also demonstrated therapeutic efficacy in melanoma, leukemia, and other solid tumors [1].

Temozolomide is a prodrug that undergoes spontaneous conversion at physiological pH to its active metabolite, 3-methyl-(triazen-1-yl) imidazole-4-carboxamide (MTIC). This intermediate further decomposes to form methylhydrazine, the active alkylating species, and 5-aminoimidazole-4-carboxamide. The antitumor activity of temozolomide is primarily attributed to DNA alkylation, particularly at the N7 position of guanine, leading to DNA damage and subsequent tumor cell apoptosis. However, some tumor cells can repair this damage through the expression

of O⁶-methylguanine-DNA methyltransferase (MGMT), thereby reducing the therapeutic efficacy of temozolomide [2–4].

A review of the literature reveals several analytical methods reported for the determination of temozolomide, including high-performance liquid chromatography (HPLC) [5,6], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [7–9], capillary electrophoresis [10], and UV–visible spectrophotometric methods using various solvents [11]. However, to the best of the authors’ knowledge, no spectrophotometric method employing MBTH as a chromogenic reagent has been reported for the determination of temozolomide in pharmaceutical formulations. Therefore, the present study aims to develop and validate a simple, sensitive, cost-effective, and selective spectrophotometric method for the quantitative determination of temozolomide in bulk drug and pharmaceutical dosage forms. The proposed method is validated in accordance with ICH guidelines and is intended for routine quality control applications.

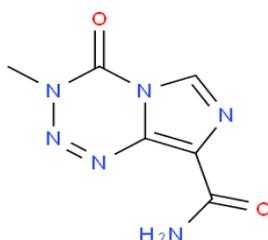


Fig-1: Chemical structure of Temozolomide

II. EXPERIMENTAL

A. Materials and Methods

A Shimadzu UV–Visible spectrophotometer (Model UV-2450) equipped with Vision Lite software was used for all spectrophotometric measurements. The instrument was operated with a spectral bandwidth of 1 nm and a wavelength accuracy of ± 0.3 nm. Quartz cells with a path length of 10 mm were used throughout the study. Spectral scanning was performed over the wavelength range of 200–800 nm at medium scan speed. All weighing operations were carried out using a calibrated electronic analytical balance (Germany). All experiments were conducted at ambient laboratory temperature (25 ± 1 °C). Pure temozolomide was obtained from Bioleo Analytical Labs India Pvt. Ltd., Plot No. 135, Oasis Towers 9, IDA Kukatpally, Prasanthi Nagar, Hyderabad, Telangana – 500072, India. 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and methanol of analytical grade were procured from SSR Enterprises, Tirupati–517501, Andhra Pradesh, India.

B. Preparation of Stock and Standard Solutions

A stock solution of temozolomide was prepared by dissolving 100 mg of the drug in 100 mL of methanol to obtain a concentration of 1000 $\mu\text{g/mL}$. From this stock solution, 10 mL was transferred into a 100 mL volumetric flask and diluted to volume with methanol to obtain a working standard solution of 100 $\mu\text{g/mL}$. Aliquots ranging from 0.5–3.0 mL of the working standard solution were transferred into a series of 10 mL volumetric flasks to obtain concentrations ranging from 5–30 $\mu\text{g/mL}$. To each flask, 1 mL of 0.5% MBTH solution was added, followed by 1 mL of 0.7% ferric chloride solution. The mixtures were gently heated, and subsequently 1 mL of 0.5 N hydrochloric acid was added. The solutions were cooled to room temperature and diluted to volume with methanol. The developed green-colored chromogen was stable for up to 32 hours. The absorbance was measured at 620 nm against a reagent blank. The concentration of temozolomide was calculated using the corresponding calibration curve.

C. Preparation of Sample Solution for Blood Analysis

Blood samples were centrifuged to separate plasma. Protein precipitation was carried out using methanol. Liquid–liquid extraction was then performed by alkalizing the plasma with 1 M sodium hydroxide, followed by extraction using 30% dichloromethane in hexane. The organic layer was separated and evaporated to dryness. The residue (100 mg) was reconstituted in 100 mL of methanol to obtain a concentration of 1000 $\mu\text{g/mL}$. From this solution, 10 mL was transferred into a 100 mL volumetric flask and diluted with methanol to obtain a working solution of 100 $\mu\text{g/mL}$. Aliquots ranging from 0.4–2.4 mL were transferred into 10 mL volumetric flasks to achieve final concentrations of 4–

24 $\mu\text{g/mL}$. To each flask, 1 mL of 0.5% MBTH solution and 1 mL of 0.7% ferric chloride solution were added. The solutions were heated, followed by the addition of 1 mL of 0.5 N hydrochloric acid. After cooling to room temperature, the solutions were made up to volume with methanol. The developed green chromogen was stable for 32 hours. Absorbance was measured at 620 nm against a reagent blank, and the concentration of temozolomide was calculated using the calibration curve.

D. Analytical Procedure

Linearity was evaluated by preparing a calibration curve using standard solutions in the concentration range of 5–30 $\mu\text{g/mL}$. The absorbance spectra were recorded over a wavelength range of 200–800 nm against the reagent blank. Maximum absorbance was observed at 620 nm, which was selected for all quantitative measurements. Precision was assessed by analyzing six replicate samples at a concentration of 20 $\mu\text{g/mL}$, and the percentage relative standard deviation (%RSD) was calculated. Accuracy was evaluated through recovery studies by spiking pre-analysed sample solutions with known amounts of pure temozolomide at 80%, 100%, and 120% levels. The percentage recovery was calculated to assess the accuracy of the method.

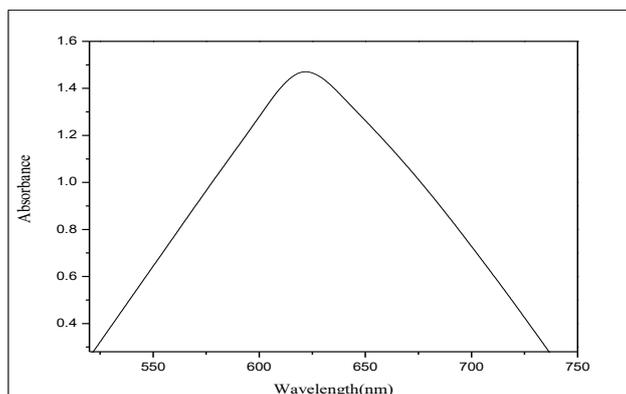


Fig-2: Absorption range of Temozolomide with MBTH/FeCl₃

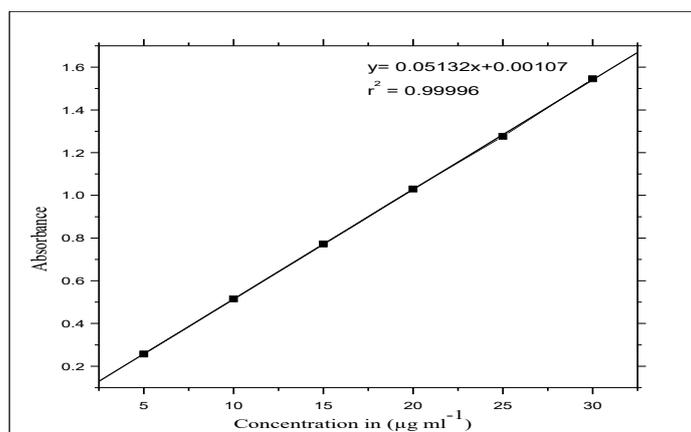


Fig-3: Beer's law plot of Temozolomide with MBTH/FeCl₃

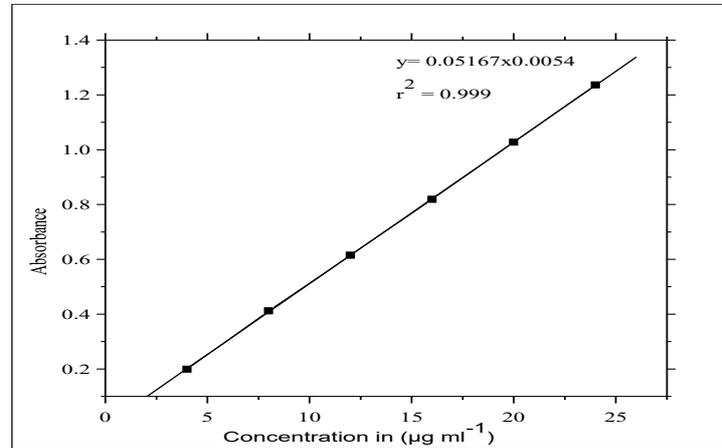
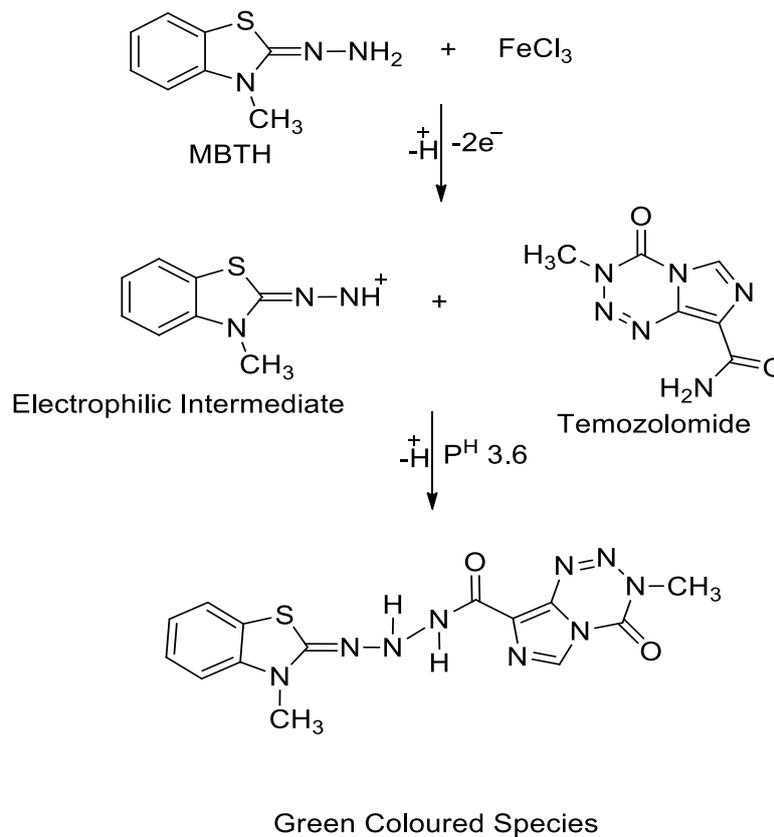


Fig-4: Beer's law plot of Temozolomide with MBTH in Blood test



Scheme 1: Schematic response system of Temozolomide with MBTH/FeCl₃

Table 1: Optical attributes of Temozolomide

Parameter	MBTH
Color	Green
Ingestion maxima (nm)	620
Brew's law limits ($\mu\text{g ml}^{-1}$)	5-30
Molar absorptivity ($\text{l mol}^{-1}\text{cm}^{-1}$)	0.0515×10^4
Sandell's Sensitivity ($\mu\text{g cm}^{-1}$)	0.647
Relapse condition (y^*)	$y=bc+a$
Incline (b)	0.05132
Intercept(a)	0.00107
Standard deviation (SD)	0.00481
Relationship coefficient (r^2)	0.99996
%RSD (Relative Standard deviation)	0.1960
LOD ($\mu\text{g ml}^{-1}$)	0.257
LOQ ($\mu\text{g ml}^{-1}$)	0.784

% RSD of six autonomous judgments

Table 2: Assay aftereffects of Temozolomide in details and Blood tests by obvious Method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed technique (mg)	Amount found by the reference strategy ¹² (mg)	% Recovery
GLIOZ	250 mg (Formulations)	249.25 $t=0.00294$ $F=5.38481$	248.95	99.5
	2mg (Blood samples)	1.97 $t=0.00291$ $F=5.2297$	1.80	98.02

- ❖ T and F-values allude to examination of the proposed technique with reference strategy.
- ❖ Theoretical esteems at 95% certainty limits $t= 0.00297$ and $F= 5.9177$ (Formulations)
- ❖ Theoretical esteems at 95% certainty limits $t=0.00269$ and $F=.5.6976$ (Blood tests)

Table-3: Determination of exactness of Temozolomide

Amount of TMZ in formulation (mg)	Amount of Standard TMZ added (mg)	Total amount found (mg)	% Recovery
249.48	200	449.06	99.06
249.80	250	44.65	99.65
249.87	300	449.76	99.76

Table-4: Determination of exactness of Temozolomide

Name of the Formulation in (mg)	Amount of Drug in Blood sample (mg)	Amount of standard drug added in (mg)	Total amount found (mg)	% Recovery
GLIOZ (2mg)	1.97	2	3.94	99.94

III. RESULTS AND DISCUSSION

The absorption spectrum of temozolomide exhibited a maximum absorbance at 620 nm, as shown in Figure 2. Calibration curves were constructed by plotting absorbance versus concentration, following Beer–Lambert’s law over the concentration ranges of 5–30 µg/mL for pharmaceutical formulations and 4–24 µg/mL for blood samples. The corresponding calibration plots are illustrated in Figures 3 and 4. The linear regression equation obtained for the method was $y = 0.05132x + 0.00107$, with a correlation coefficient ($r^2 = 0.99996$), indicating excellent linearity. The percentage relative standard deviation (%RSD) values for both precision and accuracy studies were found to be below 2%, confirming the reliability and reproducibility of the method. The optical characteristics of the method are summarized in Table 1. The percentage recovery was found to be 99.72% for pharmaceutical formulations and 99.94% for blood samples, demonstrating the high accuracy of the proposed method. The detailed recovery data are presented in Tables 2, 3, and 4. Overall, the method exhibited high sensitivity, precision, and reproducibility, confirming its suitability for routine quantitative analysis of temozolomide in pharmaceutical formulations and biological samples.

IV. CONCLUSION

The developed spectrophotometric method is simple, accurate, precise, and reliable for the quantitative determination of temozolomide in pharmaceutical formulations and blood samples. The method demonstrates excellent linearity, sensitivity, and reproducibility, making it suitable for routine quality control and analytical applications. Owing to its simplicity, cost-effectiveness, and reliability, the proposed method can be successfully employed for routine analysis in pharmaceutical laboratories.

V. ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Annamacharya Institute of Technology and Sciences, Tirupati–517520, and the Department of Chemistry, Sri Venkateswara University, Tirupati–517502, Andhra Pradesh, India, for providing the necessary research facilities. The authors also thank Analog Labs, Hyderabad, India, for supplying the drug samples used in this study.

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