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DEVELOPMENT AND VALIDATION OF UV METHOD OF TEMOZOLOMIDE IN BULK AND CAPSULE FORMULATION

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ABSTRACT: An UV spectrophotometric method for the quantitative determination of Temozolomide (TMZ) in bulk and capsule was developed in present work. The parameters linearity, precision, accuracy, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. UV spectroscopic determination was carried out at an absorption maximum of 328 nm using 0.1N Hydrochloric acid as solvent. In the UV spectroscopic method linearity over the concentration range of TMZ was found to be 2-18 µg/ml with a correlation coefficient 0.999. The limit of detection and limit of quantification were found to be 0.5271 and 1.6454 mg/ml respectively. Results of the analysis were validated statistically and by recovery studies. The proposed method is simple, rapid, precise, accurate and reliable and can be used for the routine quantitative analysis of TMZ in bulk and pharmaceutical formulation.

INTRODUCTION: Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-*as*-tetrazine-8-carbo xamide, **Fig. 1**) is an alkylating agent of the Imidazotetrazine derivatives that exhibits broad-spectrum antitumor activity against murine tumors[1]. It is a 3-methyl analogue of mitozolomide [8-carbamoyl-3-(2-chloroethyl)-imidazo-[5, 1-d]-1, 2, 3, 5-tetrazin-4-(3H)-one] which was developed as a potential alternative to dacarbazine, 5-(3-dimethyltriazen-1-yl)-imidazo-4-carboxamide (DTIC) in view of its demonstrated antitumor activity and better safety profile in preclinical assessments.

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Both and DTIC are cytotoxic alkylating agents. It has been suggested that they both exert their antitumor activity through the linear triazine, 5-(3- methyltriazen-1-yl)-imidazo-4-carboxamide [5-6]. DTIC is metabolically converted to MTIC in the liver (N-demethylation), whereas temozolomide undergoes chemical degradation to MTIC at physiological pH 6. The cytotoxicity of MTIC is thought to be primarily due to alkylation at the O^6 and N^7 positions of guanine [7-10]. In this process, MTIC itself is converted to 5(4)-aminoimidazole-4(5)-carboxamide.

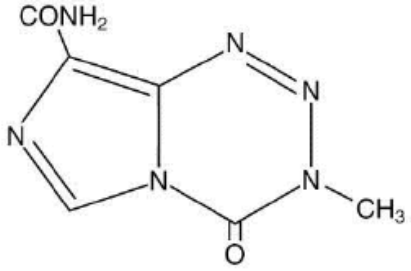

FIG. 1: CHEMICAL STRUCTURE OF TEMOZOLOMIDE

Literature review reveal there is reported a HPLC method for the analysis MTIC in human plasma [11] and stability study of Temozolomide using capillary electrophoresis [12]. However, it has the disadvantage of being time-consuming. All these studies have further emphasized the need to perform rapid and sensitive quality-control analysis of pharmaceutical formulations containing Temozolomide. As these methods are expensive, we have made an attempt to develop a more precise, simple and economical spectrophotometric method with greater precision, accuracy and sensitivity for the analysis of Temozolomide in bulk and dosage forms.

EXPERIMENTAL SECTION

Apparatus:

- Digital balance: Acculab (ALC 210.4)
- Sonicator: Eneritech (Ultra Sonicator)
- Photo stability chamber: Thermolab
- Hot air oven: Hicon
- A double beam UV-Visible spectrophotometer (Shimadzu-1800) with UV probe.

Materials, reagents and chemicals: Temozolomide bulk drug were obtained as gift sample Dr. Reddys labs Pvt. Ltd. Hyd, Capsule formulation (Temolon 5, celon) containing 100 mg obtained from local pharmacy. Hydrochloric acid, double distilled water was used throughout the analysis. All other chemicals and solvents used were of Analytical grade.

Development of Method:

Selection of solvent: Solubility of drug was performed in several solvents like ethanol, methanol, Hcl and some buffers and then UV-spectra of drugs in these solutions were recorded. Absorbances of drug were higher at distinct λ_{max} in 0.1N HCl and hence 0.1N HCl was selected as solvent for further studies.

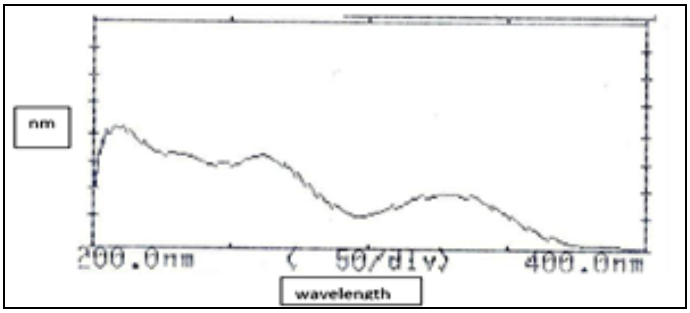

FIG. 2: U.V. SPECTRUM OF TEMOZOLOMIDE DRUG (200-400nm)

Preparation of the standard stock and calibration curve: Accurately weighed quantity of 100 mg Temozolomide reference standard was transferred into 100 ml volumetric flask and dissolved and diluted up to the mark with 0.1N Hydrochloric acid to give a stock solution having strength 1mg/ml. This stock solution used for further dilutions and by using 0.1N HCl as solvent for estimation.

The absorption maxima of temozolomide were found to be 328nm (Fig.1). Working standard solutions for the drug having concentration 2, 4, 6, 8 and up to 18µg/ml was prepared with 0.1N Hcl from the stock solution. The absorbance of resulting solutions were measured at wavelength of 328nm against solvent blank and a calibration curve was plotted to get the linearity and regression equation.

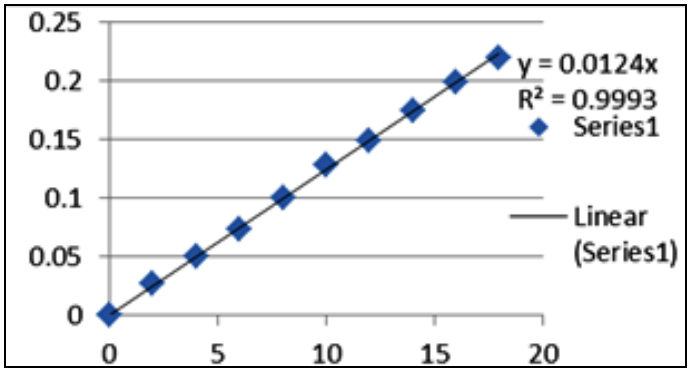

FIG. 3: CALIBRATION CURVE OF TEMOZOLOMIDE

Analysis of marketed formulation: Remove the contents of 10 capsules and weigh a quantity of powder equivalent to 100mg of Temozolomide and transfer into a 100ml standard flask. And dissolve the formulation in 0.1N hydrochloric acid solvent and allowed to sonicate for 15–20 min. Then pipette out 10ml of solution and make up to 100ml leads to 10µg/ml concentration solution. The absorbance was measured against 0.1N hydrochloric acid solvent as blank at 328 nm. The drug content was estimated by using the standard graph.

TABLE 1: ANALYSIS OF CAPSULE FORMULATIONS OF TEMOZOLOMIDE

Drug	Label claim mg/cap	Amount found	%Purity
Temozolomide	100	9.7mg	97 %w/v

Validation of the proposed method: The proposed method was validated for the following parameters.

Linearity and range: The absorbances of appropriate dilutions of standard stock solutions 2-18 µg/ mL were measured as per the developed method to confirm the linearity. The calibration curve for Temozolomide was constructed by plotting the absorbance temozolomide (Y) against concentration (X) and linearity was evaluated by linear regression equation. The slope, intercept and correlation coefficient values were recorded.

Accuracy: Accuracy is the percentage of analyte recovered by assay from known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained

Recovery studies: In order to check the accuracy and reproducibility of the proposed method, recovery studies were conducted. Recovery studies are done spiking method in this method the test sample having the concentration of 10µg/ml. to this the standard drug is spiked by adding into the test solution. Concentrations of 8, 10 and 12µg/ml are added to the sample solutions and the absorbance of the three spiked concentrations was taken. From this absorbance we can determine the amount of drug that can be recovered by the proposed method.

TABLE 2: RECOVERY STUDIES OF TEMOZOLOMIDE

Test (µg/ml)	Amount of standard drug added (µg/ml)	%Recovery	Standard deviation	%RSD
10	8	99.08	0.301	0.303
	10	99.73	0.331	0.331
	12	98.39	0.548	0.546

Precision: Precision was determined by studying the repeatability and intermediate precision. The standard deviation, coefficient of variance and standard error were calculated for the drug.

Inter-day and Intra-day precision: The intra-day concentration of the drug was calculated on the same day at an interval of two hour. Whereas the inter day concentration of drug was calculated on three different days within the laboratory conditions.

TABLE 3: TEMOZOLOMIDE INTRA-DAY AND INTER-DAY PRECISION

Temozolomide Concentration (µg/ml)	Absorbance		%RSD	
	Intra Day	Inter Day	Intra Day	Inter Day
10	**0.229** **0.225** **0.223**	**0.240** **0.232** **0.238**	**1.35**	**1.761**

Molar Absorptivity: This is the important factor for determining the absorptive property of a drug in 1 mole concentration. And this value can be useful in determining the absorbance of drug in molar concentrations. This for identifying the shifts of the maximum absorbance of the drug during the method development and the results are given in the table 1.

Limit of detection (LOD) and Limit of Quantitation (LOQ): Sensitivity of UV methods were determined from limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ of Temozolomide by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3σ /S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results are given in **table 4**.

RESULTS AND DISCUSSION: The proposed method for the determination of Temozolomide in solid dosage form was found to be precise, selective, rapid and economical. Temozolomide exhibited maximum absorption at 328nm and obeyed Beer's law in the concentration range of 2-18µg/ml. the proposed method for the determination of Temozolomide showed linear regression y = 0.01195x + 0.00406 with a correlation coefficient (R^2) of 0.999 (Figure 3). Our studies revealed a recovery percentage of 99.04, which indicates that the developed method was simple, rapid and precise. The proposed methods can be used for the drug analysis in routine quality control & method proves to be more economical than the published standard methods.

The recovery studies showed proposed method is accurate and reproducible. The results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. Accuracy, reproducibility and precision of the proposed methods were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation as shown in **Table 3**. Repeatability results indicated the precision under the same operating conditions over a short interval time and inter-assay precision. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for the method RSD is not more than 2.0 indicate good intermediate precision. The low values of LOD and LOQ, 0.5271 µg mL^{-1} and 1.6454 µg mL^{-1} for Temozolomide indicated good sensitivity of proposed method (Table 4).

Table 4: METHOD VALIDATION PARAMETERS OF TEMOZOLOMIDE

Parameters		Results
λ_{max}		328 nm
Beer's law limit		2-25 µg/ml
Molar absorptivity		66.70994 g|l
Regression equation (Y = mx + c)		y = 0.01195x + 0.00406
Slope (m)		0.01195
Intercept (c)		0.00406
Correlation coefficient (r)		0.999
Precision (%RSD)	Intra-day precision	1.306
	Inter-day precision	1.76
Accuracy(%recovery)		99.04
LOD Value (µg mL-$^{-1}$)		0.527 µg mL^{-1}
LOQ Value (µg mL^{-1})		1.6454 µg mL^{-1}

CONCLUSION: The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Thus the developed methods can be easily applied for the estimation of Temozolomide in bulk and capsule dosage form.

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