ISSN: 2347-4742

Research Article
Journal of Pharmaceutical and Biomedical Analysis Letters
www.pharmaresearchlibrary.com/jpbmal

Development and validation of UV spectrophotometric method for quantitative estimation of Temozolomide in 0.1 N HCl as a solvent

B. Mohammed Ishaq*, Hindustan Abdul Ahad, Shaik Muneer, S. Parveen, B. Fahmida

Department of Pharmaceutical Analysis, Balaji college of Pharmacy, Anantapur, A. P, India.

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 329 nm in 0.1N HCl. The percentage recovery of Temozolomide was found to be in range 98.4 - 99.92%. Beers law was obeyed in the concentration range of 2-16µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y = 0.055x + 0.033 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, glioblastoma multiforme, anaplastic astrocytoma, ICH guidelines.

Contents

Corresponding author
B. Mohammed Ishaq
E-mail: bmdishaq@yahoo.com
MS. ID: PRL2014-JPBMAL1944

PAPER-QR CODE

1. 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).

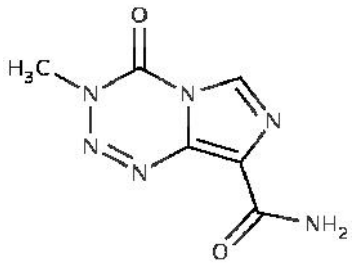

Figure 1 Chemical structures of temozolomide

Review of Literature for Temozolomide analysis revealed that several existing methods including different technique such as HPLC[2,3,4], LC/MS/MS[5,6,7], 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.

2. 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 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, Hydrochloric acid, Sodium hydroxides were purchased from SD Fine Chem, Mumbai.

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 in with 0.1N HCl 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 100mg of temozolomide capsule contents in to 100 ml volumetric flask and dissolved in 50 ml of 0.1 N HCl 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).

3. Results and Discussion

Result:

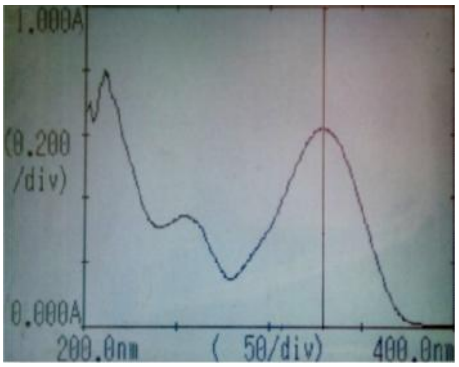

Figure.2: UV Spectrum of Temozolomide (10µg/ml) in 0.1 N HCl

Analytical method development

To develop accurate, precise and sensitive UV spectrophotometric method for temozolomide various solvent systems such as water, methanol, ethanol and 0.1 N HCl etc were tried alone and in combinations. Selection of 0.1 N HCl was based on sensitivity, minimal interference, ease of preparation, suitability for drug content estimation, stability, analysis time and cost. The λmax for temozolomide in 0.1 N HCl was found to be 329 nm (Figure 2) the method showed linear relationship (with correlation coefficient of 0.999) in the concentration range of 2-16 µg/ml (Figure 3).

Analytical method validation

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

Linearity and Range:

Various concentrations were prepared from the secondary stock solution (500µg/ml) ranging from 2-16 µg/ml. The samples were scanned in UV-VIS Spectrophotometer against 0.1 N HCl as blank. The calibration curve of temozolomide (Figure 3) was plotted between concentration of temozolomide and respective measured absorbance values at 329 nm. It was found to be linear in the specified range and the regression coefficient was found to be 0.999.

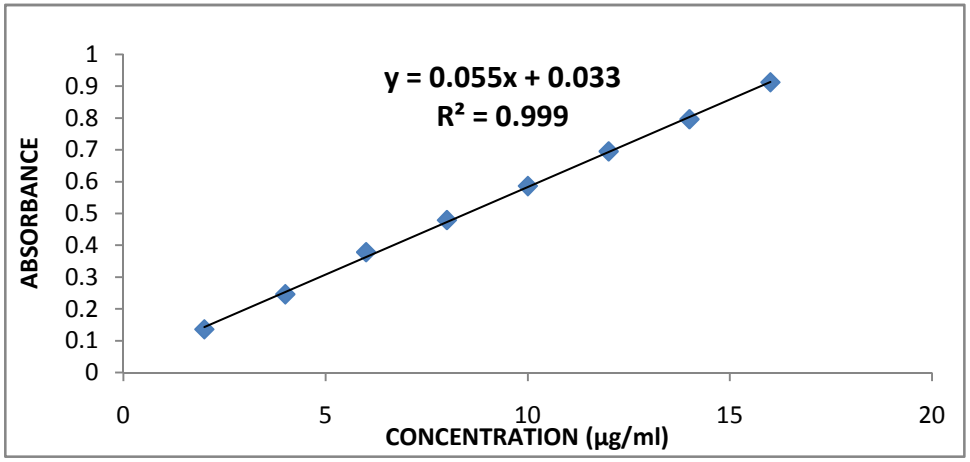

Figure.3: Calibration curve for Temozolomide in O.1 N Hcl

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 three consecutive days. The % RSD value of intraday and inter-day analysis was found to be 1.500 and 0.119 for Temozolomide (10µg/ml) in 0.1 N HCl. The results were shown in Table No 1 and 2.

Table 1: Intraday precision data for Temozolomide

Parameter	Temozolomide λ max in 0.1N HCl at 329nm	
	Standard	sample
Absorbance at λ max	0.591	0.482
	0.577	0.477
	0.575	0.4759
Mean	0.581	0.4783
SD	0.008718	0.003251
%RSD	1.500482	0.679731

Table No 2: Interday precision data for Temozolomide

Parameter	Day-1		Day-2	
	Std λ max in 0.1N HCl	Sample λ max in 0.1N HCl	Std λ max in 0.1N HCl	Sample λ max in 0.1N HCl
Day to day	0.486	0.406	0.482	0.397
	0.487	0.405	0.483	0.398
	0.486	0.404	0.484	0.394
Mean	0.4863	0.4050	0.483	0.3960
SD	0.0005	0.0010	0.0010	0.0020
%RSD	0.1186	0.2469	0.2070	0.5253

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 absorbance of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 98.4–99.92% for Temozolomide in 0.1 N HCl. The recovery data was shown in Table No 3.

Table 3: Results of recovery

Parameter Drug conc.	Absorbance in 0.1 N HCl	Amount present (µg/ml)	%Recovery (Average of three replicates)	Mean % Recovery
80%	0.546	8	98.40	99.33
	0.547			
	0.545			
100%	0.556	10	99.92	
	0.554			
	0.553			
120%	0.554	12	99.68	
	0.557			
	0.551			

Assay of marketed formulation:

The proposed method was applied to analyze commercially available Temozolomide capsules having content equivalent to 20mg. Ten capsules were weighed and powder equivalent to 100 mg transferred in 100 ml volumetric flask and dissolved in 0.1N HCl 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 0.1 N HCl 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%.

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

Table No 4: Summary of Validation parameters of Temozolomide

Parameters	Results
Beers law limit (µg/ml)	1-17
Molar absorptivity (L mol^{-1} $cm^{-1)}$	0.058
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.058
Correlation coefficient (r^2)	0.999
Regression equation (y = mx+c)	y = 0.055x + 0.033
Slope (m)	0.055
Intercept (c)	0.033
LOD (µg/ml)	0.216
LOQ (µg/ml)	0.6545
Standard Error	0.0015

Discussion:

Solubility studies were performed in different solvents such as water, methanol, 0.1N HCl, 0.1N NaOH and Phosphate buffer (pH 2). The drug was found to be freely soluble in 0.1N HCl and shown considerable absorbance values at 329nm. 0.1N HCl was taken as blank for further work. Form the stock solution (1mg/ml) different concentration as of solutions like 2-16 µg/ml were prepared and absorbance was measured at 329 nm. Calibration curve was prepared by plotting graph between absorbance vs concentration (µg/ml) (Fig1). The data was statistically validated by means of least square regression method. The detection and quantization limits were found to be 0.216

µg/ml and 654 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 98.40, 99.92 and 99.68 % 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.

4. 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.

5. Acknowledgement

The authors thank to Natco Pharma, Ltd, Hyderabad, India for providing standard Temozolomide.

6. References

1. Stevens MF, Hickman JA, Langdon SP, Chubb D, Vickers L, Stone R, Baig G, Goddard C, Gibson NW, Slack JA, *et al.* (November 1987). "Antitumor activity and pharmacokinetics in mice of 8-carbamoyl-3-methyl-imidazo [5, 1-d]-1, 2, 3, 5-tetrazin-4(3H)-one (CCRG 81045; M & B 39831), a novel drug with potential as an alternative to dacarbazine." *Cancer Res.* **47** (22): 5846–52. PMID 3664486.
2. B. Mohammed Ishaq et al., Development and Validation of a Reverse-Phase HPLC Method for Analysis of Temozolomide in a Capsule Formulation, *Int. J. Chem. Sci.: 11(2), 2013, 1055-1063*
3. Shen F, Decosterd L A, Gander M , Leyvraz S , Biollaz J , Lejeune F. Determination of temozolomide in human plasma and urine by high-performance liquid chromatography after solid-phase extraction. Journal of Chromatography, 667 (1995) 291-300.
4. Hong Kim , Paul Likhari , Donald Parker , Paul Statkevich ,Aliceann Marco , Chin-Chung Lin, Amin A. Nomeir. High-performance liquid chromatographic analysis and stability of anti-tumor agent temozolomide in human plasma. Journal of Pharmaceutical and Biomedical Analysis 24 (2001) 461–468.
5. Chowdhury S K, Laudicina D, Blumenkrantz N, Wirth M, Alton K B, An LC/MS/MS method for the quantitation of MTIC (5-(3-*N*-methyltriazen-1-yl)-imidazole-4-carboxamide), a bioconversion product of temozolomide, in rat and dog plasma. Journal of Pharmaceutical and Biomedical Analysis. 19, 5 (1999) 659-668.
6. Robert A. Parise , Mohammad Shawaqfeh, Merrill J. Egorin, Jan H. Beumer. Liquid chromatography–mass spectrometric assay for the quantitation in human plasma of ABT-888, an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase. Journal of Chromatography B, 872 (2008) 141–147.
7. Yanhong Liua, Weihua Zhangb, Yuhui Yang. Validated hydrophilic interaction LC–MS/MS method for simultaneous quantification of dacarbazine and 5-amino-4-imidazole-carboxamide in human plasma. Talanta 77 (2008) 412–421.
8. Melinda Andrasia, Rose Bustosb, Attila Gaspara, Frank A. Gomezb, Almos Klekner. Analysis and stability study of temozolomide using capillary electrophoresis.Journal of Chromatography B, 878 (2010) 1801–1808.
9. Melinda Andrasia, Brigitta Torzsoka, Almos Kleknerb, Attila Gaspar. Determination of temozolomide in serum and brain tumor with micellar electrokinetic capillary chromatography. Journal of Chromatography B, 879 (2011) 2229– 2233.
10. ICH, Q2A validation of analytical procedure,Methodology International Conference on Harmonization, Geneva, October 1994.