



Received on 23 September, 2015; received in revised form, 16 November, 2015; accepted, 05 January, 2016; published 01 March, 2016

BIO ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TEMOZOLOMIDE IN RAT PLASMA USING RP-HPLC METHOD

Swathimutyam Pallerla* and Bala Prabhakar

Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, Vile Parle, Mumbai – 400056, Maharashtra, India.

Key words:

Temozolomide,
Paracetamol, Validation, Plasma.

Correspondence to Author:

Dr. Bala Prabhakar

Associate Professor,
Shobhaben Pratapbhai Patel School
of Pharmacy and Technology
Management, Vile Parle, Mumbai –
400056, Maharashtra, India.

Email: bala.prabhakar@nmims.edu

ABSTRACT: A simple high-performance liquid chromatographic method was developed and validated for the determination of Temozolomide in rat plasma using paracetamol as internal standard. The chromatographic separation was carried out with an isocratic elution using mobile phase consisting of 0.5% glacial acetic acid and methanol. Plasma samples were run at a flow-rate of 1 ml/min using a kromasil C₁₈(4.6×250mm) column and ultraviolet detection at a wavelength of 265nm. The retention times of Temozolomide and paracetamol were found to be 6.92 and 11.2 minutes respectively. The linearity of the method was achieved over the concentration range of 10-60µg/ml demonstrated good linearity with correlation coefficient 0.999. The method was validated for accuracy, precision, linearity, recovery in compliance to international regulatory guidelines.

INTRODUCTION: Temozolomide (TMZ), an imidazotetrazine derivative, is a cytotoxic alkylating agent of broad-spectrum antitumour activity. Temozolomide was rapidly and extensively absorbed and widely distributed in tissues. It undergoes rapid chemical conversion in the systemic circulation at physiological pH to the active compound, MTIC (monomethyltriazeno imidazole carboxamide).

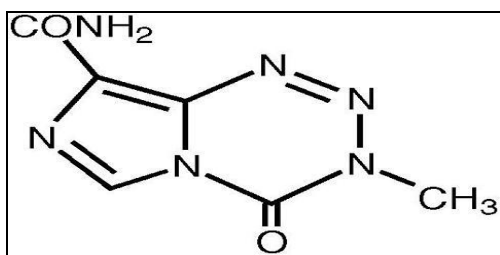
Temozolomide exhibits schedule-dependent antineoplastic activity by interfering with DNA replication. Temozolomide has demonstrated activity against recurrent glioma.

In a recent randomized trial¹, concomitant and adjuvant Temozolomide chemotherapy with radiation significantly improved, from 12.1 months to 14.6 months, progression free survival and overall survival in glioblastomamultiforme patients.

The distribution pattern of radioactivity was gender independent. Penetration into the brain following oral or intravenous administration was 10-15% based on the brain/plasma AUC ratio. Temozolomide can be taken orally, degrades spontaneously to an active metabolite and penetrates the blood-brain barrier. It is well tolerated, making it a suitable candidate for combination chemotherapy.

The aim of Present study was to validate the HPLC method for the determination of temozolomide in human plasma to allow pharmacokinetic studies in humans after the administration of TMZ^{1,2}

QUICK RESPONSE CODE	DOI: 10.13040/IJPSR.0975-8232.7(3).1298-01
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(3).1298-01	



Methyl-5-oxo- 2, 3, 4, 6, 8-pentazabicyclo [4.3.0] nona-2, 7, 9-triene- 9-carboxamide:

Chemicals and Solvents: Reference standard Temozolomide pure drug and vials were obtained from Miracalus Pharma Pvt. Ltd, Maharashtra, Mumbai, India. Internal standard paracetamol procured from Arti drugs Pvt Ltd. HPLC grade methanol, water, and acetonitrile were procured from SD Fine (Mumbai, India). Glacial acetic acid obtained from Qualigens Ltd., Mumbai.

Chromatographic Conditions:

Analysis was carried out on a HPLC system (Perkin-LC 20AT) equipped with PDA detector auto sampler, pressure controlled by prominence pump and operated by total chrom Software. Kromasil C18 column (250mm × 4.6mm particle size 5 μm) was used for separation. Mobile phase used for separation was mixture containing 0.5% glacial acetic acid and methanol (20:80 v/v). The flow rate was kept at 1.0 mL/min, column temperature was ambient (25°C), eluents were detected by PDA detector at 265 nm, and the injection volume was 20 μL.

Preparation of Standard Solution:

A standard stock solution of Temozolamide was paracetamol prepared by dissolving 100 mg drug in 100ml volumetric flask with methanol in order to make a concentration of 1mg/mL.

Preparation of 0.5% glacial acetic acid:

0.5ml glacial acetic acid was taken in 100ml volumetric flask. 100ml of water was added to make up the volume.

Preparation of Mobile Phase: Mobile phase was prepared by mixing 20 volumes of methanol and 80 volumes of 0.5% glacial acetic acid. The mobile phase was ultra sonicated, filtered through 0.45 μm membrane filter, and degassed.

Sample preparation:

Plasma spiking solution of temozolomide was prepared for calibration curve in the range of 20-100μg/ml. 90μl plasma was taken in centrifuge tube and 10 μl of standard stock solution of temozolomide and 10 μl of internal standard paracetamol has to added. 1ml methanol was used for precipitation of plasma proteins after addition of methanol vortex for 10min then centrifuged at 4000 rpm for 10min. Supernatant was transferred into another tube and evaporated under nitrogen gas for 15min. 100 μl of mobile phase was added again and centrifuged at 4000 rpm for 10min. Supernatant solution was filtered through 0.45 μ filter paper. In similar way all the standards were prepared and analysed in HPLC for calibration curve.

Selectivity:

Selectivity was evaluated by extracting different blank plasma samples. The absence of interfering peaks at the same retention time of analytes or internal standard was considered as evidence for selectivity⁴.

Linearity:

Calibration curves were constructed using linear regression within the range of 10-60μg/ml of Temozolomide.

Recovery:

Recovery of analyte was evaluated by comparing the response of temozolomide in three solvents methanol, acetonitrile and ethyl acetate.

Precision and accuracy:

For precision and accuracy studies, samples were prepared at four concentration levels, limit of quantification (LOQQC), low (LQC), medium (MQC) and high (HQC) quality controls. Corresponding to 10, 40, 60 μg/ml respectively with six replicates each. Precision and accuracy was evaluated at inter and intra batch⁵.

RESULTS:

Bio analytical HPLC method validation was developed for the quantifiable evaluation of temozolomide in plasma. Kromasil C-18 (4.6 x 250mm, 10μm) column for separation of temozolomide was used under the suitable isocratic

conditions. The chromatography conditions, especially the composition of mobile phase were optimized through several trials to achieve good resolution and symmetric peak shape for the analyte (TMZ) and internal standard. The composition of the mobile phase was optimized by varying the percentage of methanol: 0.5% glacial acetic acid (10:90), 20:80 finally 30:70 chosen as the final mobile phase since it provided the best separation, with higher sensitivity and selectivity for the UV signal of analyte and internal standard figures are shown in 1, 2, 3.

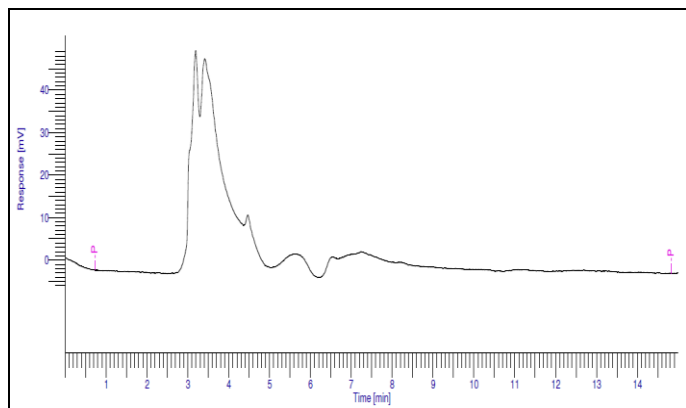


FIG. 1: BLANK PLASMA

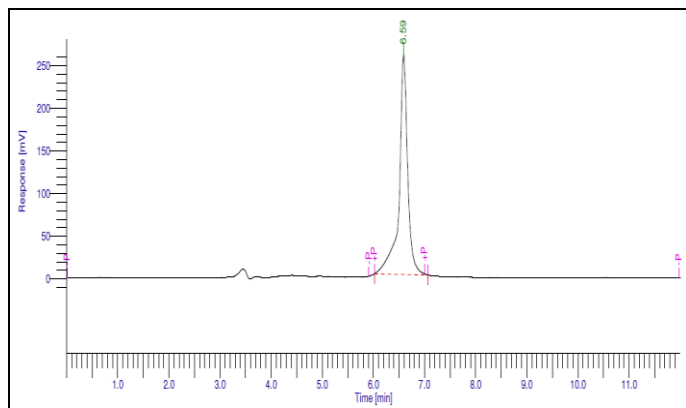


FIG 2: TEMOZOLOMIDE PEAK IN PLASMA

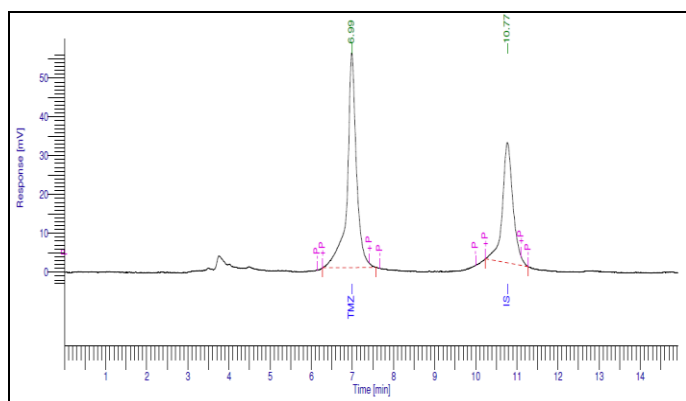


FIG 3: TEMOZOLOMIDE AND INTERNAL STANDARD IN PLASMA

TMZ calibration curve in plasma

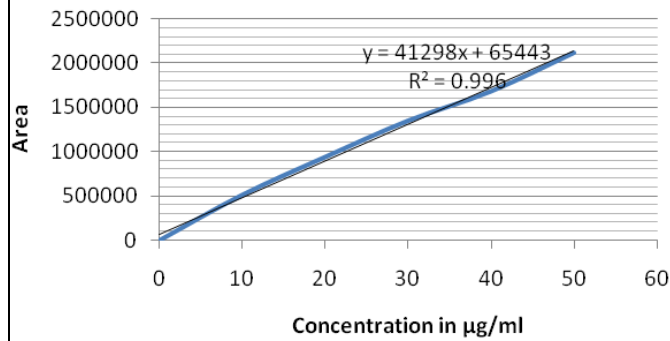


FIG 4: CALIBRATION CURVE

Selectivity:

The described method used reversed-phase HPLC for separation of temozolomide from paracetamol (internal standard) and was shown to be selective for the analyte and its internal standard (retention times for temozolomide and paracetamol were 6.92 and 11.2 minutes respectively). No interfering peaks were observed with the same retention time mentioned in **Table 1**.

TABLE 1: SPECIFICITY OF THE METHOD:

Peaks	Interference
Blank plasma	No
Blank plasma + TMZ + Internal standard	No

Linearity:

Linearity was demonstrated from 10-60 µg/ml. **Table 2** shows data from calibration curves analysed for the evaluation of precision and accuracy on different days. The calibration curve includes 8 calibration standards which are distributed throughout the calibration range. The average correlation coefficient was found to be 0.9956 with goodness of fit.

Precision:

To determine the intraday and interday precision of the method, repeatability studies were performed. The intraday and interday precision studies were carried out on two different concentrations (40 and 50 µg/mL). The samples were injected in triplicate on the same day and also on three different days. Concentration was calculated from the areas obtained, and the results were expressed as % RSD. Results are summarized in **Table 2**.

TABLE 2: SYSTEM SUITABILITY PARAMETERS

Parameters	Temozolomide
Retention time	6.98
Theoretical plates	9841
% RSD of peak area	0.98
Linearity range ($\mu\text{g/ml}$)	10-60
LOD ($\mu\text{g/ml}$)	1
LOQ ($\mu\text{g/ml}$)	5
Accuracy (%)	98.67-99.98
Precision (% RSD)	-
Intraday Precision (n=3)	0.42-0.98
Inter day Precision (n=3)	0.65-1.21
Tailing factor	1.2

Accuracy:

Accuracy of the method was assessed by three different concentrations of pure drug that were added to a known pre analysed sample, and the total concentration was determined in triplicate, and the percent recovery of the added pure drug was found in the range of 98.67 to 99.98%, indicating that the method is found to be more accurate.

CONCLUSION: The presented bio analytical HPLC method is an easy, rapid, modest, accurate, precise, specific and linear with acceptable LOD, LOQ for the determination of temozolomide in

plasma. The developed technique which is suitable for application to a pharmacokinetic, bioequivalence and drug interaction studies for the estimation of temozolomide from plasma.

REFERENCES:

1. Drug information: <http://www.Druginfo.nlm.nih.gov/>
2. Edyta G, Micha K, Anna S, Serafin B, Validated Hplc Method For Determination Of Temozolomide In Human Plasma, *Acta Poloniae Pharmaceutica N Drug Research*, 2012; 6: 1347-1355.
3. Abu B, Mohd Suhaimi A, Ahmad I, Zabidah I, Siew Hua G, Method development and validation of repaglinide in human plasma by HPLC and its application in pharmacokinetic studies, *Journal of Pharmaceutical and Biomedical Analysis*, 2014; 43(5):1831-1835.
4. Bhupinder S, Sandeep S, Naveen D, Ashish, Rama S. Bioanalytical method development and validation of alimemazine in human plasma by HPLC and its application in bioequivalence studies *Pharm Bioallied Sci.* 2013; 5(4): 257-264.
5. Kandagal P.B, Manjunatha D.H, Seetharamappa RP-HPLC Method for the Determination of Tenofovir in Pharmaceutical Formulations and Spiked Human Plasma, *Pharmaceutical Analysis*, 2013; 5: 561-570.

How to cite this article:

Pallerla S and Prabhakar B: Bio Analytical Method Development and Validation of Temozolomide in Rat Plasma Using RP-HPLC Method. *Int J Pharm Sci Res* 2016; 7(3): 1298-01.doi: 10.13040/IJPSR.0975-8232.7(3).1298-01.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)