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RP-HPLC Analysis of Temozolomide in Pharmaceutical Dosage Forms

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A simple, rapid, sensitive and precise high performance liquid chromatographic method has been developed for the estimation of temozolomide in pharmaceutical dosage forms. In this method RP-C_{18} column (250 mm × 4.6 mm I.D., 5 µm particle size) with mobile phase consisting of acetate buffer and methanol in the ratio of 75:25 v/v in isocratic mode was used. The detection wavelength is 254 nm and the flow rate is 1 mL/min. In the range of 50-300 µg/mL, the linearity of temozolomide shows a correlation coefficient of 0.9998. The percentage recovery ranges from 98.99-102.02 %. The proposed method was validated by determining sensitivity, accuracy, precision and linearity. The proposed method is simple, fast, accurate, precise and reproducible hence can be applied for routine quality control analysis of temozolomide in pharmaceutical formulations.

Key Words: Temozolomide, Validation, High performance liquid chromatographic.

INTRODUCTION

Temozolomide is an oral alkylating agent for the treatment of newly diagnosed glioblastoma multiforme and refractory anaplastic astrocytoma[1]. Temozolomide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide. The cytotoxicity of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O^6 and N^7 positions of guanine. It is chemically, 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxamide. Literature survey reveals that various high performance liquid chromatographic[2,3] and LC-MS[4] methods have been reported for the determination of temozolomide in pure and pharmaceutical dosage forms. In this study a simple, rapid, accurate, sensitive and precise high performance liquid chromatographic method was developed for the estimation of temozolomide in pharmaceutical dosage forms.

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EXPERIMENTAL

The separation was carried out on isocratic HPLC system (Waters) with Waters 1525 binary HPLC pump, waters 2487 dual absorbance detector, Waters Empower software and RP-C_{18} column (250 mm × 4.6 mm I.D.; particle size 5 µm).

Temozolomide was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade were purchased from E. Merck (India) Ltd., Mumbai. Glacial acetic acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions: The mobile phase consisting of acetate buffer (acetate buffer was prepared by dissolving glacial acetic acid in water) of AR grade and methanol (HPLC grade) were filtered through 0.45 µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 75:25 v/v was pumped into the column at a flow rate of 1 mL/min. The detection was monitored at 254 nm and the run time was 10 min. The volume of injection loop was 10 µL prior to injection of the drug solution. The column was equilibrated for atleast 0.5 h with the mobile phase flowing through the system.

Procedure: Stock solution of temozolomide was prepared by dissolving 25 mg of temozolomide in 25 mL standard volumetric flask containing 15 mL of mobile phase and the solution was sonicated for 20 min and then made upto the mark with mobile phase to get a concentration of 1 mg/mL. 1 mL of the above stock solution was transferred to 25 mL volumetric flask and the volume was made up to the mark with mobile phase. Subsequent dilutions of this solution were made with mobile phase to get concentration of 50-300 µg/mL. The solutions were injected into the 10 µL loop and the chromatogram was recorded in Fig. 1. The retention time of temozolomide was found to be 4.37 min. The calibration curve was constructed by plotting concentration *versus* peak area ratio. The amount of temozolomide present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug *versus* concentration were found to be linear and the results are furnished in Table-1.

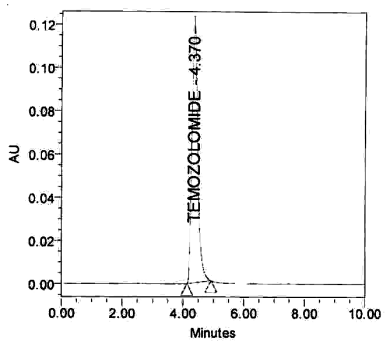

Fig. 1. Typical chromatogram of temozolomide

TABLE-1
CALIBRATION OF THE PROPOSED HPLC METHOD

Concentration (μg/mL)	Peak area ratio
50	766177
100	1552115
150	2298531
200	3124610
250	3860885
300	4597062
Slope	15395
Intercept	4936.8
Regression equation	Y = 15395X + 4936.8
Correlation coefficient	0.9998

Assay of temozolomide in capsules: Two commercial brands of capsules were chosen for testing suitability of the proposed method to estimate temozolomide in pharmaceutical dosage forms. Twenty capsules were weighed accurately. A quantity of capsule powder equivalent to 500 mg of temozolomide was weighed accurately and transferred to 500 mL volumetric flask. About 300 mL of mobile phase was added and kept in ultrasonic bath for 20 min. This solution is filtered through a membrane filter and the volume was made up to the mark with mobile phase to get 1 mg/mL concentration. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of temozolomide present in capsule formulation was determined by comparing the peak area from the standard. The results are presented in Table-2.

TABLE-2
ASSAY AND RECOVERY STUDIES

Formulation	Label claim (mg)	Amount found (mg)	Amount found (%)	Recovery (%)
Brand-1	100	99.67	99.67	99.34
Brand-2	100	99.95	99.95	100.21

Validation of proposed method: Selectivity of the method was assessed on the basis of elution of temozolomide using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of temozolomide. The results are given in Table-3.

Specificity: The specificity was established by preparing a temozolomide standard at 0.5 % level of test concentration and injected 6 times into HPLC system as per the test procedure.

Linearity: The standard curve was obtained in the concentration range of 50-300 μg/mL. The linearity was evaluated by regression analysis using the least square

TABLE-3
SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Linearity (µg/mL)	50-300
Correlation coefficient	0.9998
Theoretical plates (N)	4896
Tailing factor	1.1
Asymmetry factor	1.32
LOD (µg/mL)	0.025
LOQ (µg/mL)	0.356
Recovery (%)	98.99-102.02

method. It was found that correlation coefficient and regression analysis are within the limits.

Precision: The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (CV). The results are summarized in Table-4.

TABLE-4
PRECISION OF THE PROPOSED HPLC METHOD

Concentration of temozolomide (µg/mL)	Measured concentration of temozolomide (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 3)	C.V. (%)	Mean (n = 3)	C.V. (%)
50	50.3	0.48	50.40	0.53
100	101.7	0.67	100.25	0.61
150	151.4	0.92	149.35	0.85

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ for temozolomide were predicted on the basis of parameters of standard error of estimate and slope, calculated from linearity of the response data of temozolomide.

Accuracy: The accuracy of the HPLC method was assessed by adding known amounts of sample solutions of temozolomide at 50, 100 and 150 % of the specification were prepared in triplicate to the test solutions and injected into the HPLC system as per the proposed method. The results are furnished in Table-5.

RESULTS AND DISCUSSION

By applying the proposed method, the retention time of temozolomide in a typical chromatogram was found to be 4.37 min, which indicates a good base line. Linearity range was observed in concentration range of 50-300 µg/mL. The regression equation of temozolomide concentration over its peak area ratio was found to be $Y = 15395X + 4936.8$ ($r = 0.9998$) where Y is the peak area ratio and X is the concentration of temozolomide (µg/mL). The proposed HPLC method was also validated for intra-day and inter-day variation. When the solution containing 100 µg/mL of

TABLE-5
ACCURACY STUDIES

Concentration (%)	Amount added (µg)	Amount found (µg)	Recovery (%)	Statistical analysis	
50 Sample 1	50	49.90	99.00	Mean	100.990
50 Sample 2	50	50.19	101.96	RSD (%)	1.709
50 Sample 3	50	50.20	102.02		
100 Sample 1	100	100.01	100.08	Mean	99.940
100 Sample 2	100	99.79	98.99	RSD (%)	0.885
100 Sample 3	100	100.15	100.75		
150 Sample 1	150	149.79	99.32	Mean	100.120
150 Sample 2	150	150.11	100.37	RSD (%)	0.707
150 Sample 3	150	150.20	100.67		

temozolomide were repeatedly injected on the same day, the coefficient of variation in the peak area of drug for three replicate injections was found to be less than 1 %. The inter-day variation on three different days was also found to be less than 1 %. The asymmetry factor was found to be 1.32, which indicated asymmetric nature of peak. The number of theoretical plates was found to be 4896, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.025 and 0.356 µg/mL, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of buffer with methanol were tested. The use of buffer and methanol in the ratio of 75:25 v/v resulted in peak with good shape and resolution. The high percentage of recovery of temozolomide ranging from 98.99-102.02 % indicates that the proposed method is highly accurate.

Conclusion

The proposed HPLC method was found to be highly accurate, sensitive and precise. Therefore this method can be applied for the routine quality control analysis of temozolomide in its tablet dosage form.

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