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METHOD DEVELOPMENT AND VALIDATION OF TEMOZOLOMIDE PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A new precise, accurate, rapid method has been developed for the estimation of *Temozolomide* pharmaceutical dosage form by HPLC. From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of *Temozolomide* Educational institutions and Quality control laboratories A simple and selective HPLC method is described for the determination of *Temozolomide* Chromatographic separation was achieved on a Phenomenex C18 (250×4.6 ×5µ) using mobile phase consisting Acetonitrile : Water : Triethylamine buffer (60: 40) v/v with detection of 329 nm. Linearity was observed in the range 50-150 µg /ml for *Temozolomide* (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

Key Words: *Temozolomide,* pharmaceutical dosage form, HPLC

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.Reverse phase chromatography uses hydrophobic bonded packing, usually with an octadecyl or octyl functional group and a polar mobile phase, often a partially or fully aqueous mobile phase.Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases.Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds in table is

reversed (thus the name reverse-phase chromatography) [1-4].

Temozolomide (Temodar and Temodal) is an oral alkylating agent used for the treatment of refractory anaplastic astrocytoma -- a type of cancerous brain tumor. Temozolomide is not active until it is converted at physiologic pH to the active form, 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) [5].

Each and every day a number of diseases are being diagnosed. So, various pharmaceutical organizations are working to develop new drug molecules and new combinations of Anti-cancer drugs for better treatment. This is the reason for a greater competition in the pharmaceutical sector, and the future scenario is likely to be the same.The scope of developing and validating a method is to ensure a suitable strategy for evaluation of a particular analyte which is more specific, accurate and precise. The main focus is drawn to achieve improvement in the manufacturing and analytical conditions and making proper amendments in the standard operating procedures being followed. The above review indicates that there are fewer methods for the simultaneous estimation of *Temozolomide* but that methods was found to be cost effective and time consuming. So my aim was to develop a new method with minimum run time and less solvent consumption for the estimation of *Temozolomide* in a formulation. Hence the present study aims to develop simple, precise and accurate methods for the determination of *Temozolomide* by HPLC in tablet dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λ_{max})

Preparation of Standard solution

10 mg of Temozolomide was weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol (Fig-1).

Preparation of samples for Assay (6-8)

Preparation of Standard solution

10 mg of Temozolomide was weighed and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

Preparation of Sample solution

Weigh 20 capsules by removing the shell then crush with mortar and pestle then weigh a quantity of powder equivalent to 10mg of Temozolomide and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{\text{WS}}{DS} \times \frac{\text{DT}}{WT} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{LC} \times 100$$

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 329 nm for Temozolomide, 329 nm was selected as detector wavelength for the HPLC chromatographic method.The amount of Temozolomide present in the taken dosage form was found to be 99.35 % (Table-1).

Table-1 Results of assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
Temozolomide	20	9.85	98.5

The plate count and tailing factor results were found to be within the limits and the % RSD was found to be 0.1 so system is suitable and giving precise results (Table-2).

Table-2 Results for system suitability of Temozolomide

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.023	41587410	3285	1.2
2	3.023	41585753	3286	1.1
3	3.023	41585954	3285	1.2
4	3.023	41598374	3285	1.0
5	3.023	41598854	3285	1.1
6	3.023	41588765	3285	1.3
Mean	3.025	415908512	-	-
SD	0.0014	6112	-	-
%RSD	0.11	0.01	-	-

A graph was plotted for TEMOZOLOMIDE against the concentrations of the solutions and the peak areas (Table-3). The correlation coefficient R^2 was determined and was found to be 0.999 for Temozolomide (Fig-1)

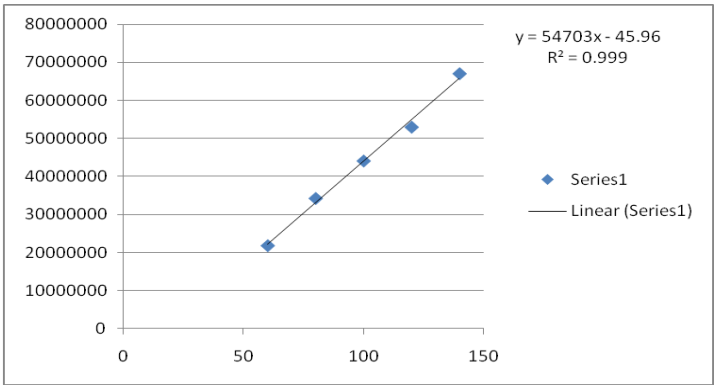
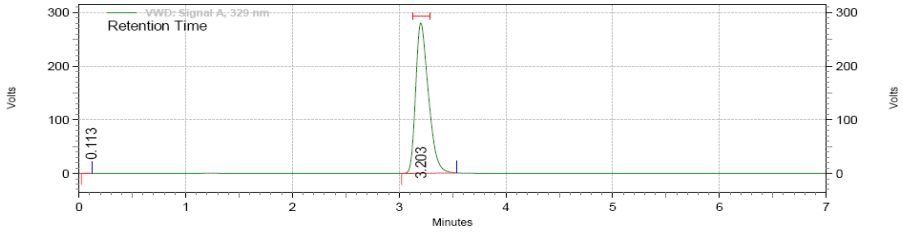


Fig-1 Graph for Linearity data of Temozolomide.

Table-3 Linearity results of Temozolomide.

S.No	Parameter	Temozolomide
1	Correlation coefficient	0.999
2	Slope	54703
3	Intercept	45.96

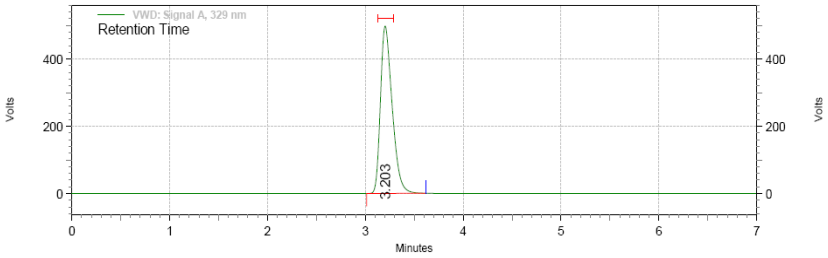
The percentage mean recovery of Temozolomide was found between 99.0 to 102.0 (Fig-2-4).



VWD: Signal A, 329 nm Results

Retention Time	Area	Area %	Height	Height %	Name
0.113	289	0.00	33	0.00	
3.203	38949327	100.00	4700514	100.00	**Temozolomide**
Totals	38949616	100.00	4700547	100.00	

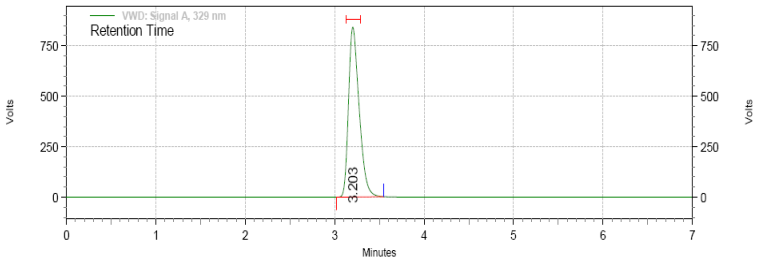
Fig- 2 Chromatogram of 50% recovery-1



VWD: Signal A, 329 nm Results

Retention Time	Area	Area %	Height	Height %	Name
3.203	69361546	100.00	8348402	100.00	**Temozolomide**
Totals	69361546	100.00	8348402	100.00	

Fig-3 Chromatogram of 100% recovery-1



VWD: Signal A, 329nm Results

Retention Time	Area	Area %	Height	Height %	Name
3.203	116439918	100.00	14104200	100.00	**Temozolomide**
Totals	116439918	100.00	14104200	100.00	

Fig-4 Chromatogram of 150% Recovery-1

CONCLUSION

A new precise, accurate, rapid method has been developed for the estimation of Temozolomide pharmaceutical dosage form by HPLC.From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Temozolomide Educational institutions and Quality control laboratories.

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