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Simultaneous Estimation of Temozolomide and Anastrozole in Capsule Dosage Form by RP-HPLC Method

P. BHARATH RATHNA KUMAR*, R. VENU PRIYA and A. VENKATESHWAR REDDY

Department of Pharmaceutical Analysis, Anwarul Uloom College of Pharmacy, Hyderabad-500 001, India

*Corresponding author: E-mail: bharathpharm@gmail.com

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A simple, rapid, accurate, specific and sensitive reverse phase HPLC method has been developed and validated for the simultaneous estimation of temozolomide and anastrozole in bulk drug and pharmaceutical capsule dosage form. The chromatographic separation was performed on the atlantis dC_{18} column (150 mm × 4.6 mm, 3 µm particle size), using a mobile phase of potassium dihydrogen phosphate pH 3 adjusted with orthophosphoric acid: acetonitrile (65:35 v/v), at a flow rate of 1 mL/min at an ambient temperature of 30 °C with the detection wavelength at 212 nm. The retention times of temozolomide and anastrozole was found to be 2.373 and 6.017 min, respectively. The proposed method has been validated for linearity, range, precision, accuracy and robustness were within the acceptance limit according to ICH Q2B guidelines. Quantification of the components in actual capsule formulations was calculated against the responses of freshly prepared external standard solutions. Linearity for anastrozole and temozolomide was found in range of 0.25-1.00 µg/mL & 25-150 µg/mL and correlation coefficient was found to be 0.999 and 0.9998, % RSD for intermediate precision was found to be 0.123 and 0.258 and for system precision 0.787 and 0.656 %, respectively. The percentage purity of temozolomide and anastrozole was found to be 99.9 and 99.83 %v/v respectively. The method was found to be robust even by change in the mobile phase ± 5 % and in less flow condition.

Keywords: Temozolomide, Anastrozole, RP-HPLC, Method development, Method validation.

INTRODUCTION

Temozolomide is a chemotherapeutic drug used to treat brain tumours called glioblastoma multiforme or anaplastic astrocytoma [1]. Temozolomide is a type of drug known as an alkylating agent and works by stopping cancer cells from making new DNA by alkylating/methylating DNA, which most often occurs at the N-7 or O-6 positions of guanine moiety. The IUPAC name of temozolomide is 4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo [4.3.0]nona-2,7,9-triene-9-carboxamide [2] (Fig. 1). Anastrozole is used in the treatment of breast cancer [3]. The IUPAC name of anastrozole is 2,2'-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropanenitrile [4] (Fig. 2). Estimation of temozolomide alone was carried out by spectroscopic methods [5-7] and HPLC methods [8,9]. Estimation of anastrozole alone was carried out by spectroscopic methods [10] and HPLC methods [11-13]. Temozolomide and anastrozole were simultaneously estimated by RP-HPLC method [14]. As per the literature survey it was felt that still more simple, rapid, accurate, specific and sensitive reverse phase HPLC method has to be developed and validated for the simultaneous estimation of temozolomide and anastrozole in bulk drug and pharmaceutical dosage form.

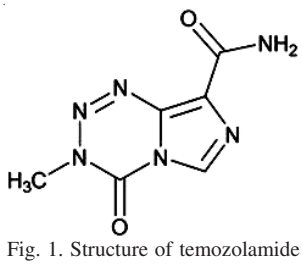

Fig. 1. Structure of temozolamide

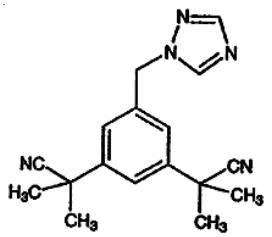

Fig. 2. Sructure of anastrozole

EXPERIMENTAL

Reference standards of temozolomide and anastrozole were supplied by Bio-Leo Labs with purity of 99.9 % and 99.95 %, respectively.

A Waters e2695 gradient system with Empower-2 software and 2489 UV/Vis detector is the most sensitive and versatile dual wavelength absorbance detector was used. It was manufactured by the company Waters, Alliance HPLC systems, Japan. Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used.

Chromatographic conditions: The column used was Atlantis d C18 Column (150 mm × 4.6 mm, 3 µm particle size) was used for analytical separation. The mobile phase consisted of potassium dihydrogen phosphate (pH 3) and acetonitrile in the ratio of (65:35 % v/v). The flow was adjusted to 1 mL/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 212 nm. The isobestic point showing in Fig. 3 purity analysis was performed over a wavelength range of 200-400 nm. The injection volume was 20 µL capacity.

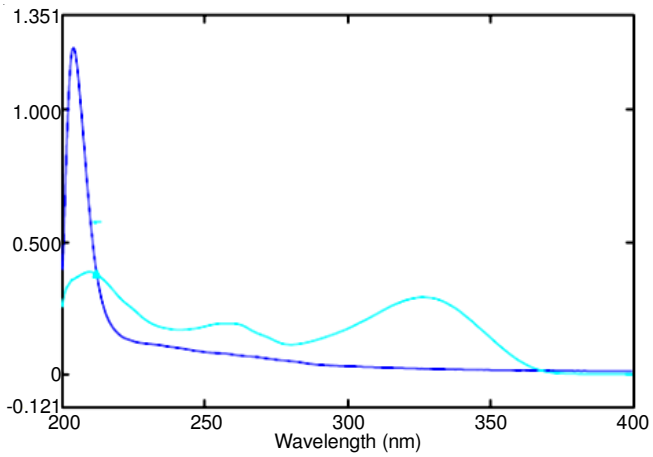

Fig. 3. Isobestic point of anastrozole and temozolamide

Preparation of analytical solutions

Preparation of potassium dihydrogen phosphate buffer solution: A weighed quantity of 5.444 g of potassium dihydrogen phosphate (KH_2PO_4) taken in a 1000 mL beaker. To this add 400 mL of HPLC water and mixed in ultra Sonicator and filtered through 0.45 µ membrane filter and the resulting solution is having the pH 3.

Preparation of mobile phase: Mix a mixture of above buffer 650 mL (65 %), 350 mL of acetonitrile (HPLC grade-35 %) and degas in ultrasonic water bath for 5 min. Filter through 0.45 µ filter under vacuum filtration.

Preparation of anastrozole and temozolomide standard solution: About 1 mg anastrozole and 20 mg temozolomide were accurately weighed, transferred to 100 mL volumetric flask and dissolved in mobile phase. The volume was made up to the mark with mobile phase to get the working standard solution (10 µg/mL for anastrozole and 200 µg/mL for temozolomide).

Preparation of sample solution: Twenty capsules were taken and an amount of powder equivalent to 1 and 20 mg each of anastrozole and temozolomide was taken in a 100 mL volumetric flask and made up the volume with diluting solvent. Further the solution was filtered through 0.45 µ membrane filter. Several aliquots of standard anastrozole and temozolomide stock solution were taken in different 100 mL volumetric flasks and diluted upto the mark with mobile phase so that the final concentrations of anastrozole and temozolomide were in the range of 0.25-1.50 µg/mL and 25-150 µg/mL, respectively.

Method development and validation of HPLC: The suggested analytical method was validated according to ICH guidelines [15] with respect to certain parameters such as specificity, linearity, precision, accuracy, robustness and system suitability.

Specificity: The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced degradation studies are carried out by using 0.1 M HCl, 0.1 M NaOH, heat and UV light.

Linearity: Linearity of the method was evaluated at different concentration levels by diluting the standard anastrozole and temozolomide solutions to give solutions over the range 0.25-1.50 µg/mL and 25-150 µg/mL for both anastrozole and temozolomide. The first level and last level were injected in six replicates and % RSD was calculated for these runs and rests of levels were injected in duplicate. Concentrations were input into a Microsoft Excel® spreadsheet program to plot calibration curves. Limit of detection and limit of quantitation to calculate limits of detection (LOD) and limits of quantification (LOQ) values, sequential dilutions prepared and analyzed by the proposed method. The LOD and LOQ established by evaluating the level (signal to noise ratio of 3:1 and 10:1 respectively) at which the analytes can be readily detected and quantified with accuracy

Precision (repeatability): Precision of the assay was demonstrated by injecting six different sample solutions containing anastrozole equivalent to 0.25-1.50 µg/mL and temozolomide equivalent to 25-150 µg/mL and % RSD was calculated

Accuracy: Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80, 100 and 120 % for anastrozole and temozolomide. Standard and sample solutions are injected in to HPLC system in triplicate and percentage recoveries of anastrozole and temozolomide were calculated. The area of each level was used for calculation of % recovery.

Robustness: Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase changed from 1.0 to 0.9 and 1.1 mL/min. The temperature of the column was changed from 25 to 30 and 35 °C. The solutions described for robustness study were applied on the column in triplicate and the responses were determined.

System suitability: System suitability tests were carried out on freshly prepared standard stock solutions of anastrozole and temozolomide and it was calculated by injecting standards in six replicates at 6 min interval and the values were recorded.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of anastrozole and temozolomide. The method developed was proceeding with wavelength selection. The optimized wavelength was 212 nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From

several trials final method is optimized with the following conditions.

The mobile phase consisted of an aqueous solution of potassium dihydrogen phosphate buffer (pH 3) and acetonitrile in the ratio of 65:35 % v/v and the column used was Atlantis d C18 Column (150 mm × 4.6 mm, 3 µm particle size). The flow rate was adjusted to 1 mL/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 212nm and purity analysis was performed over a wavelength range of 200-400 nm. The injection volume was 20 µL.

The specificity of the method was to determine whether there are any interference of any impurities in retention time of analytical peak.

The linearity was determined as linearity regression of the claimed analyte concentration of the range 0.25-1.50 µg/mL (anastrozole) and 25-150 µg/mL (temozolomide). The calibration curve obtained by plotting peak area *versus* concentration was linear and the correlation coefficient was found to be 0.9989 and 0.999 for temozolomide and anastrozole respectively (Table-1).

TABLE-1
LINEARITY RESULTS FOR
TEMOZOLOMIDE AND ANASTROZOLE

S. No.	Anastrozole		Temozolomide	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
1	0.25	305153	25.00	939041
2	0.50	664510	50.00	2047315
3	0.75	948707	75.00	2903443
4	1.00	1288825	100.00	3963308
5	1.25	1656474	125.00	5086560
6	1.50	1989557	150.00	5993745

Precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The % relative standard deviation for system precision was found to be 0.656 and 0.787, respectively (Table-2). The % relative standard deviation for method precision was found to be 99.949 and 0.999, respectively (Table-3).

The accuracy study was performed in 80 %, 100 % and 120 %. The percentage recovery was determined for temozolomide and anastrozole was found to be 99.83 % and 99.9 % presented in Tables 4 and 5.

The robustness were carried out with minor but deliberate changes in parameters *i.e.*, detection wavelength, column temperature and flow rate (Table-6). Theoretical plates and tailing factor were observed and were found to be 3819.10 and 13183.12 (theoretical plates) and 1.12 and 0.97 (tailing factor) for temozolomide and anastrozole respectively. The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than

TABLE-2
SYSTEM PRECISION VALUES FOR
TEMOZOLOMIDE AND ANASTROZOLE

S. No.	Temozolamide		Anastrozole	
	RT	Area	RT	Area
1	2.37	4126457	6.006	1328169
2	2.361	4125678	5.996	1315689
3	2.37	4103697	5.998	1308169
4	2.352	4157635	5.941	1324763
5	2.361	4126358	5.962	1300289
6	2.365	4179354	5.974	1317896
Avg	2.363	4136530	5.980	1315829
Std Dev	0.0068	27129.40	0.0250	10359.07
RSD	0.288	0.656	0.419	0.787

TABLE-3
METHOD PRECISION VALUES FOR
TEMOZOLOMIDE AND ANASTROZOLE

S. No.	Temozolamide		Anastrozole	
	RT	Area	RT	Area
1	2.369	3982589	6.001	1295120
2	2.368	3975231	5.998	1296358
3	2.37	3991258	5.997	1297421
4	2.367	3962014	5.998	1298473
5	2.371	3982587	5.996	1299357
6	2.372	3971047	5.998	1298697
Avg	2.370	3977454	5.998	1297571
Std Dev	0.0019	10272.79	0.0017	1598.28
RSD	0.079	0.258	0.028	0.123

TABLE-4
RECOVERY STUDIES FOR TEMOZOLOMIDE

Conc. (%) (at specification level)	Area	Amount added (mg)	Amount found (mg)	Recovery (%)	Mean recovery
80	3168377	80	79.891	99.86	
100	3963837	100	99.949	99.95	99.83
120	4744206	120	119.63	99.69	

TABLE-5
RECOVERY STUDIES FOR ANASTROZOLE

Conc. (%) (at specification level)	Area	Amount added (mg)	Amount found (mg)	Recovery (%)	Mean recovery
80	1031015	0.8	0.7997	99.97	
100	1288393	1.0	0.9990	99.94	99.9
120	1545293	1.2	1.1990	99.89	

TABLE-6
LIST OF ROBUSTNESS VALUES FOR TEMOZOLOMIDE AND ANASTROZOLE

Parameters	Flow rate	Average area		RT	
		Temozolomide	Anastrozole	Temozolomide	Anastrozole
Flow rate	0.9 mL/min	5185425	1669446	2.56	6.387
	1.0 mL/min	3978951	1298602	2.373	5.995
	1.1 mL/min	4347307	1405266	2.179	5.420
Temperature	25	4738302	1510422	2.356	6.387
	30	3978351	1298602	2.373	5.995
	35	4869445	1583855	2.349	5.420

2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in Table-7.

TABLE-7
SYSTEM SUITABILITY PARAMETERS FOR TEMOZOLOMIDE AND ANASTROZOLE

S. No.	Parameters	Temozolomide	Anastrozole
1	Average area	4136530	1315829
2	Retention time	2.363	5.980
3	Tailing factor	1.12	0.94
4	USP plate count	4005.68	12025.63

Conclusion

A new method was established for simultaneous estimation of temozolomide and anastrozole by RP-HPLC method. The instrument used was WATERS- HPLC Auto sampler, separation module 2695, UV detector 2487, Empower-software version-2. The chromatographic conditions were successfully developed for the separation of temozolomide and anastrozole by using atlantis dC_{18} (150 × 4.6 mm ID), 3 µm particle size, flow rate was 1.0mL/min, mobile phase ratio was (65:35 %v/v). Potassium dihydrogen phosphate:acetonitrile, detection wavelength was 212nm.The retention times were found to be 2.373 and 6.017 min. The % purity was found to be 99.9 % & 99.83 % v/v respectively. The system suitability parameters for temozolomide and anastrozole such as theoretical plates and tailing factor were found to be 3819.10 & 13183.12 and 1.12 & 0.97 and the resolution was found to be 19.86. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for temozolomide and anastrozole was found in concentration range of 0.25-1.5 µg/mL & 25-150 µg/mL and correlation coefficient (r^2) was found to be 0.999 and 0.9989, % recovery was found to be 99.9 % to 99.83 % and % RSD for system precision was 0.787 & 0.656, % RSD for repeatability % RSD for intermediate precision was 0.123 & 0.258 respectively. The study was precise, robust, repeatable. LOD value was 0.10193 and, 0.22097 LOQ value was 0.30888 and 0.6696.

Hence the suggested RP-HPLC method can be used for routine analysis of temozolomide and anastrozole in API and Pharmaceutical dosage form.

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