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Method Development and Validation of a Reversed Phase HPLC Method for Determination of Anastrazole and Temozolomide in Pharmaceutical Dosage Form

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Abstract. A new simple assay method has been developed and validated for the determination of Anastrazole and Temozolomide using reverse-phase high-performance liquid chromatography in their pharmaceutical dosage form. The chromatographic separation was performed on an Inertsil ODS (4.6 x 150 mm, 5 m) using mobile phase phosphate buffer pH 3.0 and methanol of 30:70% v/v at a flow rate of 0.8 mL/min. Analytes were detected at 260 nm. The method was found to be linear in the concentration range of 1-5 µg/mL for both medicaments with the coefficient value (R2) of >0.999. The accuracy was measured via recovery studies and found to be acceptable and the percentage recoveries were found in the range of 98.81-100.720 and 99.290-100.700%. The proposed method was successfully validated and applied for the quantitative estimation of these drugs in both bulk and tablet dosage forms.

INTRODUCTION

Anastrazole is a potent aromatase inhibitor and treats advanced breast cancer [1-8] in women who have gone through "the change of life" (menopause) [9] and administered by mouth. Anastrozole works by lowering estrogen hormone levels to help shrink tumors and slow their growth. Anastrozole has been tested for reducing estrogens, including estradiol, in men [10]. It can also contribute to decreasing the risk of stroke, heart attack, chronic inflammation, prostate enlargement and prostate cancer [11]. Chemically it can be represented as 2-[3-(1-cyano-1-methyl-ethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl]-2-methyl-propanenitrile (Figure 1) with formula of $C_{17}H_{19}N_5$, mass was 293.36 g/mol. The physicochemical properties are white crystalline solid, odorless and are freely soluble in methanol, acetone, ethanol, and tetrahydrofuran and very soluble in acetonitrile1,2 having melting point 81-82 ^{0}C. Temozolomide (8-carbamoyl-3-methyl-imidazo- [5, 1-d]-1, 2, 3, 5-tetrazin-4-(3*H*)-one(12,13,14) (Figure 2) with molecular formula $C_6H_6N_6O_2$, is an alkylating agent of the imidazotetrazine derivatives that exhibits broad-spectrum antitumor activity against murine tumors. It was rapidly and extensively absorbed and widely distributed in tissues and undergoes fast chemical conversion in the systemic circulation at physiological pH to the active compound, MTIC (monomethyltriazeno imidazole carboxamide). A literature survey revealed that there are various analytical methods such as UV [12], HPLC [13-15] and LC-MS [16,17] which were reported for the estimation of individual drugs and in combination with other drugs. However, to the best of our knowledge, a simple, rapid method has not been published for the above-mentioned components. The reason for selecting this combination is to provide a

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validated HPLC method which can separate two different drugs in a single formulation that may be useful in the pharmaceutical industry.

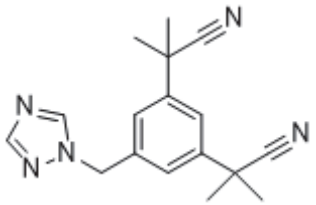

FIGURE 1. Structure of Anastrazole

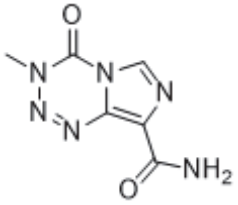

FIGURE 2. Structure of Temozolomide

RESULTS AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

The main objective of this study was to develop and validate an assay method for the simultaneous estimation of Anastrazole and Temozolomide by reverse-phase high-performance liquid chromatography. Several chromatographic trials were conducted using various solvents such as methanol, ACN, water, and different phosphate buffer pH levels at different ratios. During the mobile phase selection, it was found that buffer could help in separating two drugs with good resolution. The best results were achieved by using the Inertsil C18 (4.6 x 250mm, 5m) with the mobile phase consisted of phosphate buffer (pH 3.0) and methanol in the ratio of 30:70% v/v at 260 nm using a PDA detector. The retention time of Anastrazole and Temozolomide was found to be 2.569 and 3.842 min, respectively. The optimized chromatograms are given in Figure 3.

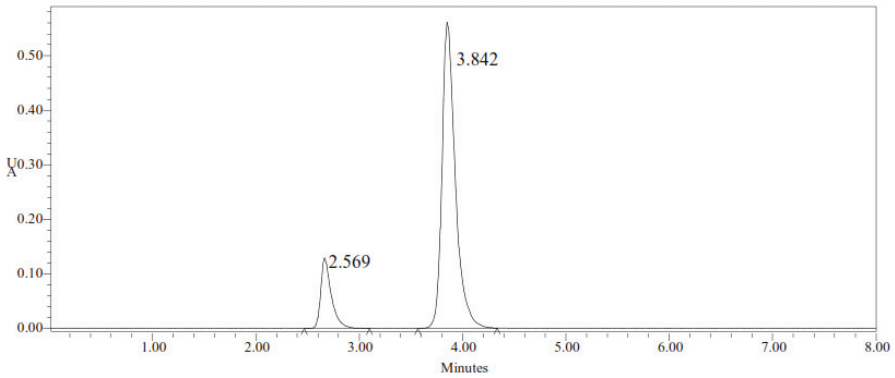

FIGURE 3. Chromatogram for Anastrozole and Temozolomide

Analytical Method Validation

The proposed method was validated according to the ICH guidelines [17] for specificity, recovery, precision, linearity, system suitability, robustness, the limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

Linearity, LOD, and LOQ

The linearity of an analytical method was evaluated over the concentration range of standard solutions ranging between 1-5 µg/mL were prepared for both medications and their peak areas were recorded. The linearity of the calibration curve was checked by constructing a plot of area versus concentration. The LOD and LOQ were measured from the calibration curve method. The Detection limit and quantification limit value of Anastrazole and Temozolomide was found to be 0.29, 0.07 and 0.88, 0.22. The statistical linearity data are presented in Table 1 and linearity data represented in Figure 4 and Figure 5.

TABLE 1. Statistical data of calibration curve

Parameter	**Anastrazole**	**Temozolomide**
Linearity range	1-5 µg/mL	1-5 µg/mL
Regression equation	y = 30029x + 37313	y = 28447x + 38353
Limit of detection	0.29 µg/mL	0.07 µg/mL
Limit of quantification	0.88 µg/mL	0.22 µg/mL

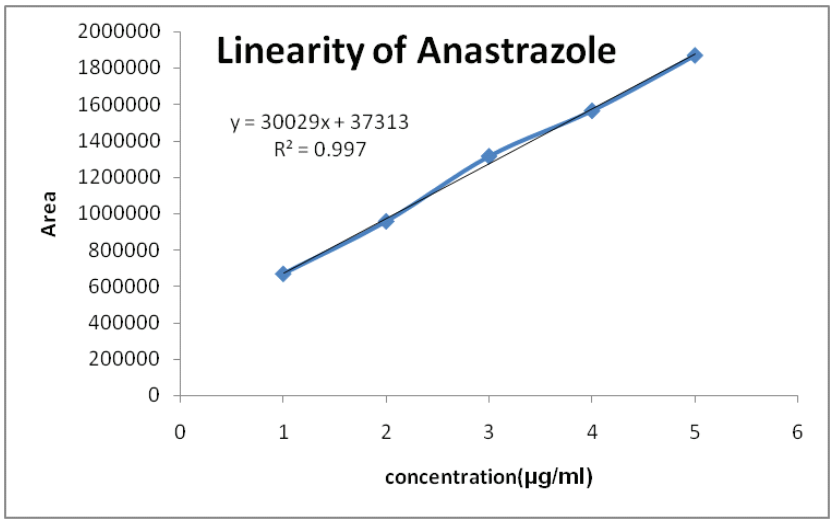

FIGURE 4. Linearity graph of Anastrazole

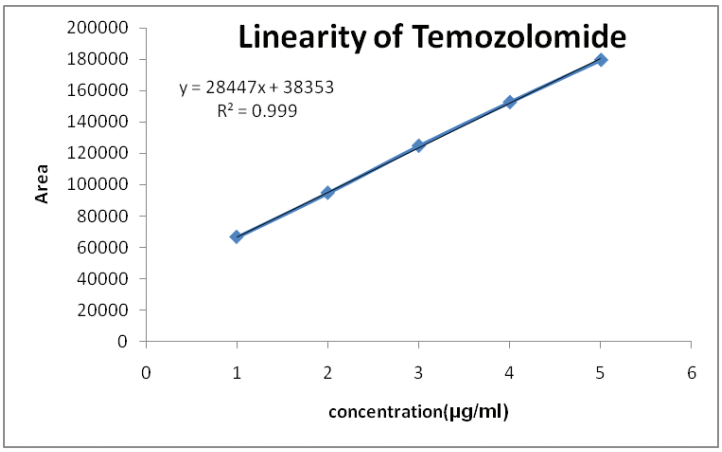

FIGURE 5. Linearity graph of Temozolomide

Precision

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Anastrazole (3 µg/mL) and Temozolomide (3 µg/mL) have been analyzed by injecting them into an HPLC column on the same day and on consecutive days. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in Table 2.

TABLE 2. Results of intra-day and inter-day precision

Sample	**Concentration**	**Mean area**		**% RSD**	
	(µg/mL)	**Intra-day**	**Inter-day**	**Intra-day**	**Inter-day**
Anastrazole	3	1304529.8	124162.7	0.2	0.6
Temozolomide	3	1305070.2	122681.8	0.2	0.1

Recovery

The percentage recovery was calculated by preparing standard drug concentrations of Anastrazole and Temozolomide with concentration levels of 50%, 100%, and 150%. A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration and the mean percentage recovery of Anastrazole and Temozolomide was achieved between 98.81-100.720 and 99.290-100.700%. The results are given in Table 3.

TABLE 3. Recovery Study of Anastrazole and Temozolomide

Compound	**Quantity(mg/ml)**		**Mean % recovery**
	Amount added	**Amount found**	
Anastrazole	5	5.036	100.720
	10	10.003	10.003
	15	14.822	98.813
Temozolomide	5	5.035	100.700
	10	10.001	100.010
	15	14.894	99.290

Robustness

Robustness of the proposed analytical method was a measure of its capacity to remain unaffected and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (0.8 ± 0.2 mL) and 10% mobile phase ratio. The parameters chosen for the study of robustness were the flow rate and mobile phase. From the results obtained, there were no significant changes observed at the end of the study.

Analysis of Tablet Dosage Form

The developed method was applied for the estimation of drugs in the commercial tablet dosage form (Anastrazole 50 mg/ Temozolomide 0.5 mg tablets). The chromatograms show the good separation of the samples with acceptable limits such as tailing factor and USP plate counts. The % assay was calculated and found to be satisfactory and the results are given in Table IV.

TABLE 4. Analysis of tablet dosage form

Drug	Dosage (mg)	Amount found (mg)	% Assay
Anastrozole	50	49.975	99.95
Temozolomide	0.5	0.5012	100.24

SUMMARY

A rapid, specific, and reliable isocratic reversed-phase high-performance liquid chromatographic method with UV detection has been developed and validated for the determination of ANA and TOZ in pharmaceutical formulations. The method involves the use of a simple mobile phase and minimum sample preparation, encouraging its application for quality control of ANA and TOZ in tablets.

EXPERIMENTAL SECTION

Reference materials of Anastrazole were supplied by Mylon, Temozolomide from Cipla, HPLC grade methanol and water (Merck), Acetonitrile (Molychem), Orthophosphoric acid (Merck) and samples of tablets were procured from the local market. The mobile phase and solvents were prepared using potassium dihydrogen orthophosphate (98.0%) and HPLC grade methanol. The analysis was performed on Waters HPLC, autosampler and PDA detector. Data were together and evaluated by empowering software and analyte elution by inertsil ODS (4.6 x 150mm, 5m) as a stationary phase with ambient temperature program, a solvent mixture of phosphate buffer pH 3 Methanol (30:70%v/v) at detection of 260 nm. All the drugs and chemicals were weighed on Afcoset ER-200A electronic balance, pH meter (Adwa-AD 1020) and a sonicator (Frontline FS 4, Mumbai, India). The mobile phase was degassed by ultrasonic vibrations prior to use.

Instrument and Chromatographic Separation

Chromatography was performed on a WATERS 2695 HPLC column (Waters Corporation, Milford, USA) with an autosampler with PDA detector. Components were detected at 260 nm and data processing was achieved by Empower 2 software. The chromatographic separation was performed on Inertsil C18 (4.6 x 250mm, 5m) column at an ambient column temperature. The samples were eluted using phosphate buffer (pH 3): methanol (30:70% v/v) as the mobile phase at a flow rate of 1 mL/min. The mobile phase was filtered through a 0.45-µm nylon filter and it was degassed before use. 10 µL of sample solutions were injected into the HPLC system.

Standard Solution Preparation

Standard and working solutions Anastrazole (10 mg) and Temozolomide (10 mg) were accurately weighed and transferred into a 10-mL clean and dry volumetric flask separately and 7 mL of the diluent was added. It was sonicated for 30 min and diluted to the final volume with the diluent. From the above stock solution, 0.3 mL of the solution was transferred into another 10 mL volumetric flask and then diluted to the final volume with the diluent.

Sample Solution Preparation

Twenty tablets were weighed and powdered finely and the weight of the powder equivalent to 10 mg was transferred into a 10 mL volumetric flask and 7 mL of the diluent was added, the flask was sonicated for 25 min and the volume was made up with the diluent and filtered. Finally, 0.3 mL of the filtered solution was pipetted out, transferred into a 10 mL volumetric flask and made up the final volume with diluents.

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REFERENCES

1. P. E. Lonning, Endocr. Related Cancer. **11**, 179-189 (2004).
2. A. Howell, J. Cuzick, M. Baum, A. Buzdar, M. Dowsett, J. F. Forbes, G. Hoctin-Boes, J. Houghton, G. Y. Locker, J. S. Tobias, Lancet. **365**, 60-62 (2005).
3. A. G. Murugesan, S. Sasi premila, K. Bala Amutha, J. Comput. Method. Mol. Design. **1**, 1-10 (2011).
4. P. S. Sukhramani, S. R. Tirthani, S. A. Desai, M. P. Suthar, Der Pharmacia Lett. **3**, 236-243 (2011).
5. A. Mohammad, F. Bano Faruqi, J. Mustafa, Arch. Appl. Sci. Res. **1**, 178-199 (2009).
6. M. A. Aweda, K. K. Ketiku, A. T. Ajekigbe, A. Edi, Arch. Appl. Sci. Res. **2**, 300-312 (2010).
7. S. Malgounda Pati, H. Prakash Joshi, Der Pharmacia Lett. **4**, 961-967 (2012).
8. A. D. Agrawal, S. R. Bavaskar, Y. M. Bagad, M. R. Bhurat, Der Pharmacia Lett. **2**, 338-345 (2010).
9. B. Z. Leder, J. L. Rohrer, S. D. Rubin, J. Gallo, C. Longcope, J. Gallo, C. Longcope, J. Clin. Endocrinol. Metab. **89**, 1174-1180 (2004).
10. N. Mauras, K. O. OBrien, K. O. Klein, V. Hayes, J. Clin. Endocrinol. Metab. **85**, 2370-2377 (2000).
11. P. V. Plourde, M Dyroff, M. Dukes, Breast Cancer Res Treat. **30**, 103-111 (1994).
12. M. Banerjee, D. Tejaswini Kumari, K. Ankita, K. Swapneswar, Der Pharma Chemica. **6**, 140-144 (2014).
13. B. K. Patel, R. H. Parikh, J. Chem. Pharma. Res. **8**, 258-263 (2016).
14. B. Mohammed Ishaq, K. Vanitha Prakash, G. Krishnamohan, Int. J. Chem. Sci. **11**, 1055-1063 (2013).
15. G. Saravanan, M. Ravikumar, M. J. Jadhav, M. V. Suryanarayana, N. Someswararao, P. V. R. Acharyulu, Chromatogr. B. **66**, 291-294 (2007).
16. D. Vinayak, H. Ganesh, Y. Ravi, M. D. Rokade, Biol. Forum. **4**, 55-60 (2012).
17. ICH guidelines for validation of analytical procedure (1996): Methodology and Harmonized Tripartite Guideline. Q2B.