Abstract

Quality is important in every product or service, but it is vital in medicine as it involves life. Unlike other consumer goods, there can be and there is no second quality. Therefore analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, are a stable and will continue to meet purported quality throughout its shelf life.

These methods should be selective and sensitive to monitor the known and unknown impurities, have to be written in a format such that they can be produced over a period of time and from laboratory to laboratory, i.e. these methods should be validated.

Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B). The US Food and Drug Administration (FDA) and US Pharmacopoeia (USP) both refer to ICH guidelines, i.e. these methods should be validated.

The specific aim was to develop a method, which can be used simultaneously for the estimation of Anastrozole & Temozolomide and also for the estimation of their related compounds and degradation products in bulk as well as in marketed formulations. Temozolomide and Anastrozole are not official in any pharmacopoeia and not a single analytical method was reported for the simultaneous estimation of these drugs in the formulation and Related compounds using the same method.

For the present studies analytical methods based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), and Mass Spectroscopy (LC-MS) have been developed.
From the Literature review it is unable to find the combined HPLC methods for their respective formulations of Temozolomide and Anastrozole are present in publications however, an analytical methods for individual analysis for Temozolomide and Anastrozole was published. It is felt necessary that to develop quantitative LC method for simultaneous determination of Temozolomide and Anastrozole for their respective formulations. It was also found that there are some analytical methods reported for the Temozolomide and Anastrozole separately and most of the works reported were done on the biological fluids.

A fast, sensitive and accurate reverse phase liquid chromatographic method was developed and validated for the simultaneous determination of Anastrozole and Temozolomide in an anticancer dosage form. Chromatographic separation was achieved on Inertsil C8 column (250 X 4.6 mm, 5 µm particle size) with a separate gradient mobile phase consisting of Ammonium acetate buffer and Acetonitrile with at a flow rate of 1.0 mL/ min. The analytes were detected at 215 nm for Anastrozole and Temozolomide by PDA detector. The retention time of Temozolomide and Anastrozole were found to be at 7.663 ± 0.1 and 23.158 ± 0.1 mins respectively. The linearity of the method ranged between 0.0165 and 15.0 µg/ mL for both the Anastrozole and Temozolomide with correlation coefficient 0.999 for both the drugs in a binary mixture. The LOD was found to be 0.00825 µg/ mL and 0.0085 µg/ mL for Anastrozole and Temozolomide respectively and LOQ was found to be 0.0165 µg/ mL and 0.017 µg/ mL for Anastrozole and Temozolomide respectively.

For Related substances method validation, The proposed method found within the specified criteria for system suitability, Method found specific and selective as all the impurities of Anastrozole and Temozolomide found separated, also peak purity found with homogenous peaks.
In linearity chapter all the correlation coefficient found more than 0.99 for Anastrozole, Temozolomide and their respective impurities, In Accuracy chapter the recovery found Between 85.0% to 115.0%. Comparison results of precision and intermediate precision study found within the limit as absolute difference is within acceptance criteria and Results found within the acceptance criteria for Robustness study
Peak purity of main peak in all conditions of force degradation passes.PDA Scan for degraded drug substance and drug product is comparable to that of untreated drug substance and drug product. All peaks due to degradation are well separated from each other and from main peak. So there is no interference of blank, placebo and degradant at retention time of main peak.
The proposed LC-MS/MS method can be regarded as selective, accurate, precise, and valid for determination of anastrozole with a total running time of 2.0 min. Through this method it was possible to evaluate, anastrozole quantification in human plasma and offers advantages over methods previously reported. The present method is more sensitive, while the analytical run is shorter, permitting a high throughput.
The advantages of the proposed method involve a simple procedure for sample preparation and relatively short time of analysis. Apart from this, it can be used for assays of Anastrozole and Temozolomide in biological fluids or in pharmacokinetic investigations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection, and limit of quantitation. Robustness and stability of solutions. The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Anastrozole and Temozolomide.
It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard Pharmacopeial methods for the simultaneous determination of Anastrozole and Temozolomide using the HPLC systems with PDA detector.