RP-HPLC estimation of Capecitabine in pure and pharmaceutical dosage form

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ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatographic method was developed for the estimation of capecitabine in pure and pharmaceutical formulations. Capecitabine was chromatographed on a reverse phase C18 column in a mobile phase consisting of Ammonium acetate buffer and acetonitrile in the ratio 65:35 v/v. The mobile phase was pumped at a flow rate of 1.2 mL/min with detection at 240 nm. The detector response was linear in the concentration of 20-120 µg/mL. The limit of detection and limit of quantification was found to be 0.0026 µg/mL and 0.0088 µg/mL, respectively. The intra and inter day variation was found to be less than 1%. The mean recovery of the drug was 99.82%. The proposed method is simple, fast, accurate, precise and reproducible hence can be applied for routine quality control analysis of capecitabine in bulk and pharmaceutical formulations.

Keywords: Capecitabine, RP-HPLC, tablets, validation.

INTRODUCTION

Capecitabine, [N4-pentoxycarbonyl- 5- deoxy-5-fluorocytidine] is an anticancer prodrug of 5- fluorouracil (5-FU) that was designed to undergo preferential conversion to 5-FU within tumors1-2. Literature survey revealed HPLC3-13 and spectrophotometric methods14-15. This paper presents simple, rapid, reproducible and economical method for Capecitabine by RP-HPLC in tablet dosage form.

EXPERIMENTAL

Instruments used
HPLC experiments were performed on a Shimadzu HPLC system equipped with Phenomenex Luna C18, 5µm (4.6x250 mm) column, two LC-20AD pumps, SCL-10AVP system controller, SIL-20A auto injector, SPD-20A UV-visible detector and LC Solution software was used. Injection volume was 10 µL. The mobile phase consisted of ammonium acetate buffer and acetonitrile in the ratio 65:35 v/v that was set at a flow rate of 1.2 mL/min.

Preparation of mobile phase:
650mL of ammonium acetate buffer and 350mL of acetonitrile was mixed in a suitable container for 1000mL of mobile phase, filtered through 0.45µm porosity membrane filter and stored.

Preparation of ammonium acetate buffer
1.540 g of ammonium acetate was weighed and transferred into a 1000 mL volumetric flask and dissolved in small amount of water for HPLC and made up to the volume with the same.

Preparation of drug stock solution
Stock solution of capecitabine was prepared by dissolving accurately weighed 100 mg of the drug in 100 mL of mobile phase (1 mg/mL). The prepared stock solution was stored at 4°C protected from light.

Selection of analytical wavelength
By appropriate dilution of standard stock solution with mobile phase, various concentrations of capecitabine were prepared separately. The solutions were scanned using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. The λmax of capecitabine was found to be 240 nm which was selected as the analytical wavelength for further analysis.

Calibration standards
Calibration plot (fig.1) was constructed by analysis of appropriate working solutions (concentration 20, 40, 60, 80, 100 and 120 µg/mL) of capecitabine in the mobile phase and plotting concentration against peak-area response for each injection. Unknown samples were quantified by reference to these calibration plots.

Fig. 1: Calibration curve for capecitabine.

Assay
The tablets were chosen for testing suitability of the proposed method to estimate capecitabine in pharmaceutical formulations. Tablets were weighed accurately and powdered. A quantity equivalent to 100 mg of capecitabine was weighed accurately and transferred to 100 mL volumetric flask with mobile phase. The contents were sonicated for 20 min, and made up to the mark with the mobile phase. The resulting solution is filtered through a membrane filter. 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 capecitabine present in tablet formulation was determined by comparing the peak area from the standard.

**Validation**

Validation of the developed method was done by performing the linearity, LOD, LOQ, precision and accuracy studies.

**RESULTS AND DISCUSSIONS**

The mobile phase was fixed based on trial and error method. The mobile phase consisted of ammonium acetate buffer and acetonitrile in the ratio 65:35 v/v that was found suitable for elution of the drug at a flow rate of 1.2 mL/min. Blank chromatogram is given in fig. 2. Chromatogram for standard solution is given in fig. 3. System precision was done by injecting a standard solution and peak area responses for five replicate injection of the standard solution were recorded (Table 1). The standard curve was obtained in the concentration range of 20-120 µg/mL (Table 2). The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits. The proposed method was applied for the tablets and accuracy studies were carried out (Table 3). The limit of detection (LOD) and limit of quantification (LOQ) for capecitabine were found to be 0.0026 µg/mL and 0.0088 µg/mL respectively.

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 (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table 3.

**CONCLUSION**

The proposed RP-HPLC method was found to be simple, specific, precise, accurate, rapid and economical for assay of capecitabine in individual tablet dosage form. This method was validated as per ICH guidelines. The sample recoveries were in good agreement with their respective label claims and suggested non-interference of formulation excipients in the method. Hence, this method can be easily and conveniently adopted for routine analysis of capecitabine in tablet dosage form.

**REFERENCES**

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