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Validated, reversed phase high performance liquid chromatography method for the estimation of capecitabine in pharmaceutical formulations

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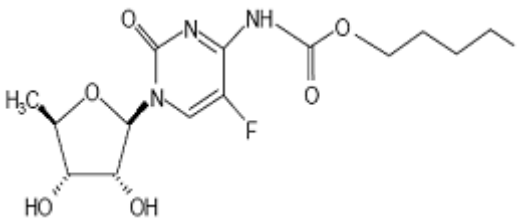
ABSTRACT

Reversed phase high performance liquid chromatographic method was developed and validated for estimation of Capecitabine in tablet dosage form. A Phenomenex Prodgy C18, 150x4.6 mm i.d, 5 µm partical size, with mobile phase consisting of mixed buffer of 0.005 M potassium dihydrogen orthophosphate and 0.005 M dipotassium hydrogen orthophosphate (pH 6.8) and acetonitrile in the ratio of 70:30 v/v was used. The flow rate was 1.0 ml/min and the effluents were monitored at 240 nm. The retention time was 5.89 min. The detector response was linear in the concentration of 25-300 mcg/ml, with the regression coefficient of 0.9999. Quantification was done by calculating area of the peak and the detection and quantitation limits were 0.125 and 0.375 mcg/ml respectively. The percentage assay of Capecitabine was 99.91%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method can be applied for the determination of Capecitabine in quality control samples and formulations without interferences of the excipients present.

Key words: Capecitabine, RP-HPLC, Estimation, and Tablets.

INTRODUCTION

Capecitabine[1] is a novel antineoplastic agent; with a chemical name PentylN-[1-[(2R, 3R,4R,5R)-3,4-dihydroxy-5-methyl-oxolan-2-yl]-5-fluoro-2-oxo-pyrimidin-4-yl]carbamate, with a molecular formula $C_{15}H_{22}FN_3O_6$ and a molecular weight of 359.39. Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity indicated for the treatment of patients with metastatic breast cancer. It is an orally administered systemic prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) which is converted to 5-fluorouracil. Literature survey reveals few Chromatographic methods[2-4] for the determination of Capecitabine in biological matrix like plasma etc. So far, no assay procedure has been reported for the estimation of Capecitabine from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Capecitabine in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Capecitabine in bulk drug samples and in pharmaceutical dosage form.



Structure of Capecitabine

EXPERIMENTAL

Materials and methods

Capecitabine was obtained as a gift sample from Shilpa Medicare Ltd, Raichur,

Karnataka State. Potassium dihydrogen orthophosphate and Dipotassium hydrogen orthophosphate were of analytical grade, and supplied by M/s S.D.Fine Chem Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Capecitabine tablets (Capiibine, Dr.Reddy's-500 mg) were procured from local Pharmacy.

Instrument

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 100 µl, and 2693 pump. A RP C-18 Phenomenex Prodgy column (150×4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions

The mobile phase consisting of mixed buffer of 0.005 M potassium dihydrogen orthophosphate and 0.005 M dipotassium hydrogen orthophosphate (pH 6.8) and acetonitrile in the ratio of 70:30 v/v. The mobile phase was filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 10.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 240 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 50 mg of Capecitabine in 50 ml volumetric flask containing 30 ml of diluent (50:50 v/v acetonitrile: water), sonicated for about 15 min and then made up to 50 ml with diluent to get a 1mg/ml standard stock solution

Working Standard solution

12.5 ml of the above stock solution was taken in 50 ml volumetric flask and thereafter made up to 50 ml with diluent to get a concentration of 250 µg/ml.

Preparation of Sample solution

Ten tablets (Capiibine, Dr.Reddy's-500 mg) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 50 mg of the active ingredient, was mixed with 25 ml of diluent. The contents of the flask was sonicated to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding diluent to obtain a stock solution of 1.0 mg/ml. An aliquot of this solution was transferred to a 10 ml volumetric flask and made up to sufficient volume with mobile phase to give a concentration of 250 mcg/ml.

Linearity

Aliquots of standard Capecitabine stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of Capecitabine

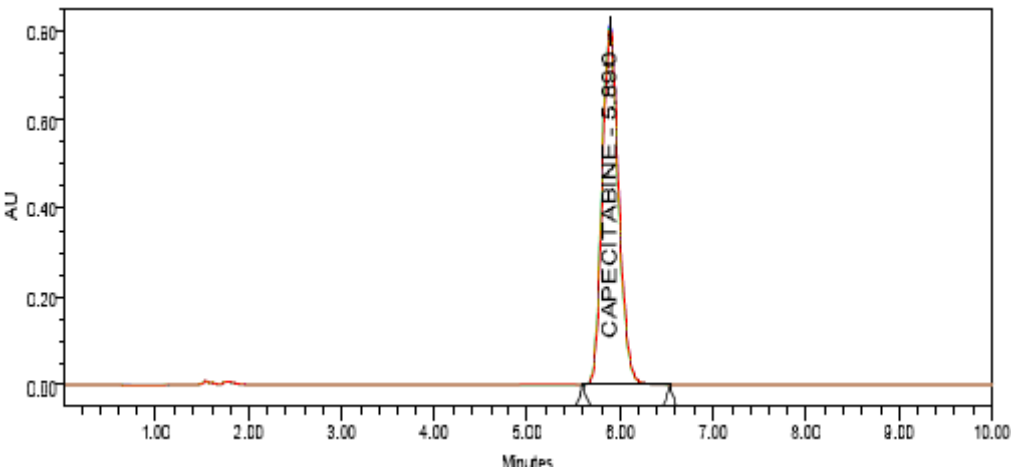


Fig 1: Typical Chromatogram of Capecitabine by HPLC

are in the range of 25-300 mcg/ml Each of these drug solutions (20 µL) was injected three time into the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 240 nm and a Calibration graph was obtained by plotting peak area versus concentration of Capecitabine (Fig 2).

The plot of peak area of each sample against respective concentration of Capecitabine was found to be linear in the range of 25–300 mcg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being Y= 39480x + 129100. The regression characteristics, such as slope, intercept, standard deviation on slope (Sa), the standard deviation of the intercept (Sb), and %RSD were calculated for this method and given in Table 1.

Table 1: Linear regression data for calibration curves

Drug	Capecitabine
Concentration range (mcg/ml)	25-300
Slope (m)	39480.0
Standard deviation on slope (Sm)	$4.4x10^2$
Intercept (b)	129100
Standard deviation on intercept (Sb)	$1.04x10^5$
Correlation coefficient	0.9999
% RSD	0.27

Assay

20 µl of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 5.89 mins. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery Studies

Accuracy was determined by recovery studies of Capecitabine, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of

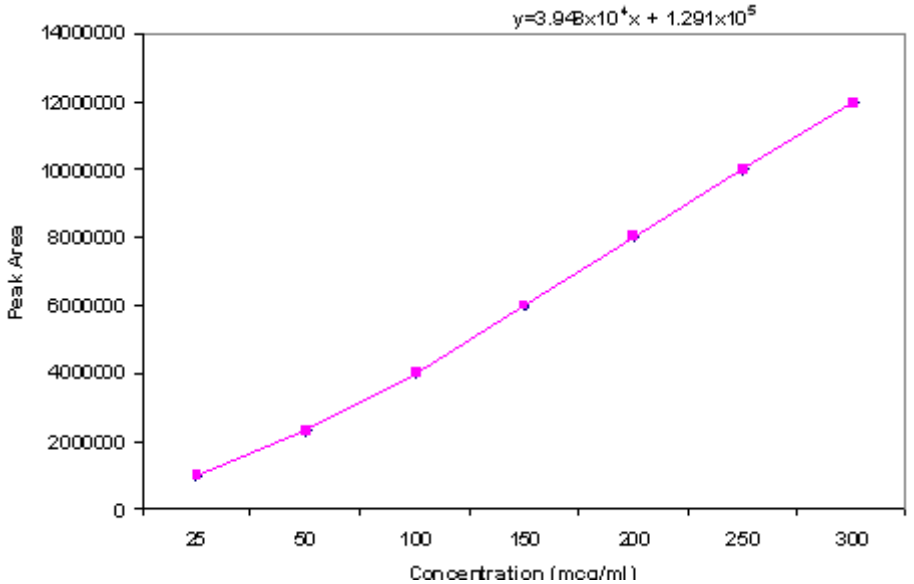


Fig 2: Calibration curve of Capecitabine by HPLC

Table 2: Results of HPLC assay and Recovery studies

Sample	Amount claim (mg/tablet)	% found by the proposed method	% Recovery*
1.	500	99.58	101.25
2.	500	100.52	101.50
3.	500	99.65	99.84

*Average of three different concentration levels.

Table 3: Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	5072
Tailing factor	1.15
Retention time in minutes	5.89
LOD (mcg/ml)	0.125
LOQ (mcg/ml)	0.375

Capecitabine. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

Limit of detection (lod) and limit of quantification (loq)

The limit of detection (LOD) and limit of quantification (LOQ) for Capecitabine were found to be 0.125 and 0.375 µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

From the typical chromatogram of Capecitabine as shown in fig 1, it was found that the retention time was 5.89 min. A mixture of mixed buffer of 0.005 M potassium dihydrogen orthophosphate and 0.005 M dipotassium hydrogen orthophosphate (pH 6.8) was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 25-300 mcg/ml. Low values of standard deviation are indicative of the high precision of the method. The assay of Capecitabine tablets was found to be 99.91%. From the recovery studies it was found that about 100.86% of Capecitabine was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of Capecitabine within a short analys is time.

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