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A new validated RP-HPLC method for the estimation of Capecitabine in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple and accurate RP-HPLC method has been developed for the estimation of Capecitabine in bulk and pharmaceutical dosage forms, using C_8 Hypersil BDS (Base Deactivated Silane) 250 X 4.6 X 5μ particle size in isocratic mode, with mobile phase comprising of buffer (pH 2.3-2.5) and Acetonitrile in the ratio of 80:40 v/v. The flow rate was 1.2 ml/min and the detection was monitored out by UV detector at 240nm. The retention time for Capecitabine was found to be 5.825min.The proposed method has permitted the quantification of Capecitabine over linearity in the range of 20-120 μg/ml and its percentage recovery was found to be 99.96-100.32 %. The intra day and inter day precision were found 0.16% and 0.10%, respectively.

Key words: Capecitabine, HPLC, Isocratic.

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INTRODUCTION

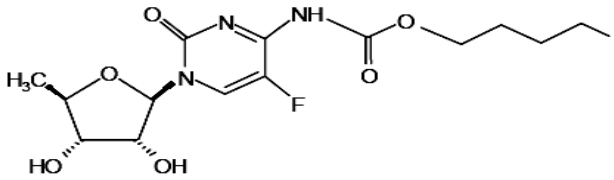

Fig.1. Chemical structure of Capecitabine

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity and it is in a class drugs known as anti metabolites. The chemical structure of Capecitabine was shown in fig.1. Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR), which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. The activation of capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR), to form 5-fluorouracil. Chemically it is 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] - cytidine with empirical formula of $C_{15}H_{22}FN_3O_6$ and the molecular weight of 359.35 g/mol. It elicits the pharmacodynamic response by resembling as a normal cell nutrient needed by cancer cells to grow. The cancer cells take up the Capecitabine, which then interferes with their growth. The length of treatment depends on the types of drugs you are taking, how well your body responds to them, and the type of cancer you have. Literature review [1-5] reveals that few analytical methods have been evoked for the estimation of Capecitabine in human plasma and some of the estimations by HPLC were reported. In present study the authors were developed a sensitive, accurate and reliable method for the estimation of Capecitabine in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Reagents & materials

Pure standard of Capecitabine (99.32%) was obtained from Dr.Reddy's Pharmaceutical Pvt.ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile (Ranchem), HPLC grade water, Sodium dihydrogen phthalate (Merck), Methanol (Merck), Xabine Capsules (Ranbaxy), Electronic analytical balance (Sortorius, Metler to ledo AT261, Delta Range), Sonicator (Enertech), Micro pipette (In labs, 10-100µl), pH meter (Elico-11610) were employed in the study. All the glassware employed in the work washed with hot water, followed acetic anhydride then acetone and dried in hot air oven and utilized when ever required. Working environment was maintained in between 18-22^{0}c. How ever, the chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC, and XRD studies.

HPLC apparatus and chromatographic conditions

The HPLC system (Shimadzu co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp , A Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 240nm), A Shimadzu model C-R5A chromatograph integrator module (chart speed at 10mm/min and an attenuation 0), A Shimadzu model SIL-6A auto injector and A Shimadzu module SCL-6A system controller. Isocratic elution of mobile phase (80:40 v/v of buffer and Acetonitrile) with flow rate of 1.2 ml/min was performed on C_8 Hypersil BDS analytical column, 5µm; 250x4.6mm i.d. Integration of the detector out put was performed using the Shimadzu class Vp soft ware to determine the peak area. The contents of the mobile phase were 80:40 v/v Buffer pH (2.3-2.5) and Acetonitrile. They were filtered through 0.45 µm membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.2 ml / min which yield a

column back pressure of 100-120 kg/cm^2. The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was detected at 240 nm.

Preparation of mobile phase

Buffer pH (2.3-2.5) and Acetonitrile in the ratio of 80:40 v/v were employed as a mobile phase and Buffer solution was prepared as directed by the procedure of Indian pharmacopoeia (1996).

Preparation of stock solution of Capecitabine

A stock solution was prepared by dissolving 100mg of Standard Capecitabine in a 100 ml volumetric flask containing 70 ml of methanol (HPLC grade) and sonicated for about 15 min and the volume made to the mark with methanol. Daily working standard solutions of Capecitabine were prepared by suitable dilution of the stock solution with the mobile phase where, five sets of analyte solution were prepared in the mobile phase containing Capecitabine at a concentration of 40-120 µg/ml. Each of the these dilutions (20µl) was injected six times in to the column, with flow rate of 1.2 ml/min and peak area of each of the drug concentrations, retention times were recorded.

Construction of linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (1- 3 ml) and diluted up to 25 ml to get the desired concentrations for linearity in the range of 20-120 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times into the column. The calibration curve for Capecitabine was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 40-120 µg/ml with good correlation in between concentration and mean peak area.

Estimation of Capecitabine in Capsule dosage form

20 Capsules were weighed and the contents were removed to obtain the average weight powder. A sample of the powder claimed to contain 100 mg of active ingredient, was mixed with 70 ml of methanol and allowed to sonicate for 20 minutes then make up the volume up to 100ml with methanol with mixing. Take above potion of the test solution and centrifuged at 2500 RPM for ten minutes. Further the resulting solution was passed through 0.45 µm membrane filter followed by adding of methanol to obtain a stock solution of 1mg/ml. An aliquot of this solution (0.4 ml) was transferred to a 10 ml volumetric flask and made up to a sufficient volume with mobile phase to get desired concentration of 40 µg/ml. The prepared dilution was injected five times in to the column to obtain chromatogram. From that peak area, the drug content in the capsules was quantified.

RESULTS AND DISCUSSION

Method development

The present RP – HPLC method for the quantification of Capecitabine in bulk and pharmaceutical dosage forms, revealed as simple, accurate and precise method with significant retention time of 5.825min. Buffer pH (2.3-25) and Acetonitrile in the ratio of 80:40 v/v were employed as mobile phase. The typical chromatogram of Capecitabine was shown in fig.2.

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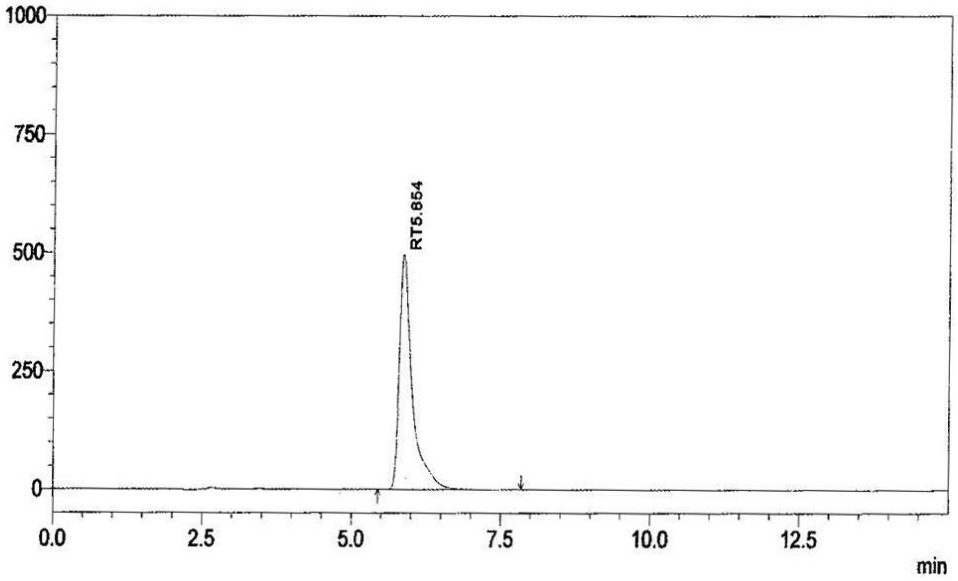

Fig.2. A typical Chromatogram of Capecitabine

Method validation

Linearity

The linearity for the detection of Capecitabine was 20-120µg/ml with (R^2= 0.994; Y=16047x-83096) the coefficients of variation based on mean peak area for the five replicate injections were found to be 0.06 to 0.63. Results were shown in table-1 and statistical data of calibration curves were shown in table-2.

Table.1. Concentration Vs Mean Peak area of Capecitabine

Concentration(µg/ml)	Mean peak area*	%RSD
20	3052621	0.63
30	4795541	0.52
40	6564200	0.48
50	7989751	0.12
60	9621831	0.53
70	10258778	0.26
80	12962558	0.06
90	14002675	0.14
100	16576432	0.23

*Mean of five values, Regression equation (y= 16047x-83069), R^2= 0.994

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Table.2. Statistical Data of Calibration Curves of Capecitabine

Parameters	Capecitabine
Linearity	20-100µg/ml
Regression equation	16047x-83096
Standard deviation of slope	0.017
Relative standard deviation of slope (%)	0.435
Standard deviation of intercept	0.333
Correlation coefficient (r^2)	0.994

Precision of the method

The intraday and inter-day variations of the method were determined using five replicate injections of three concentrations and analysed on the same day and three different days over a period of two weeks. The result revealed the precision with %RSD (0.16% and 0.10%) respectively for intra day and inter day. Results were shown in table–3.

Table.3. Intra and inter day precision of Capecitabine

Concentration (µg/ml)	Observed concentration*			
	Intra day	%RSD	Inter day	%RSD
20	20.02	0.16	20.04	0.10
30	30.03	0.14	29.98	0.17
40	39.98	0.13	39.04	0.16

*Mean of five values

Accuracy of the method

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting five times at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of capecitabine standard to a sample of known concentration and calculating the recovery of Capecitabine with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 99.96-100.32% and were given in table -4.

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Table.4. Recovery Studies of Capecitabine

S.No.	Sample	Wt. of sample per ml (mg)	Amount of standard added per ml (mg)	Amount of standard recovery per ml (mg)	Percentage Recovery
1	Sample + 80%	0.2035	0.0064134	0.006433	100.31
2	Sample + 100%	0.2035	0.080168	0.008014	99.96
3	Sample + 120%	0.2035	0.0096202	0.009651	100.32

Estimation of Capecitabine in capsule formulation

The assay for the marketed tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.006 of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in table-5.

Table.5. Amount Capecitabine in capsule dosage form

Brand name	Labeled Claim (mg)	Amount found*±S.d	%Purity ±S.d
Xabine	500	500.03±0.02	100.006±0.26

*Mean of five values

System suitability

To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values were shown in table-6.

Table.6. System suitability parameters

Retention time	5.802
Theoretical Plates	3889.97
Tailing factor	1.8
Linearity Range (µg /ml)	20-120
Limit of Detection (LOD) (µg /ml)	0.090
Limit Of Quantitation (LOQ) (µg /ml)	0.402
Relative standard deviation (RSD)	0.606

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Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the Capecitabine as per the proposed method and each dilution injected to different column and analyst on different days. The results were shown in table-7.

Table.7. Ruggedness (Method Precision)

		Amount estimated					
S.no	Labeled claim	Analyst-1	Analyst-2	System-1	System-2	Column-1	Column-2
1	100	101.2	100.9	99.7	101.8	99.6	101.2
2	100	100.1	100.3	98.6	100.5	98.2	99.8
3	100	100.1	100.0	98.5	100.7	98.3	100.4
4	100	99.9	100.0	99.1	99.2	99.1	99.4
5	100	99.8	100.0	98.4	99.8	98.4	100.2
6	100	100.4	99.8	98.3	100.4	98.3	100.2
Mean±%RSD		100.24±0.6	100.16±0.5	98.8±0.5	100.2±0.5	98.8±0.4	100.5±0.3

Robustness

Robustness of the proposed method was estimated by taking the different percentage of organic phase(Acetonitrile) composition (90%,100%,110%) of mobile phase, Changing the column brand, by maintaining different flow rates (0.8,1.0,1.2) and by maintaining the different column temperatures (28^0c,30^0c,32^0_c). The System suitability parameters were established and found to be within acceptable limits. The Results were shown in table-8. The proposed method indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table. 8. Robustness of the method

System suitability parameters (RSD of Capecitabine peak area from Six replicate injections of standard preparation)	Variation in the mobile phase composition			Variation in the flow rate			Variation in the column temperature		
	90%	100%	110%	0.8	1.0	1.2	28^0c	30^0c	32^0c
	0.2	0.4	0.2	0.1	0.2	0.1	0.2	0.2	0.3

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Detection and quantification limits

Limits of Detection (LOD) and Quantification (LOQ), the limits of detection and quantification on were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Specificity

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

CONCLUSION

The results of the study reveal that the proposed RP-HPLC method for the estimation of Capecitabine is simple and accurate in bulk and pharmaceutical dosage forms.

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