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Research Article

DEVELOPMENT AND VALIDATION OF NEW RP HPLC METHOD FOR ANALYSIS OF CAPECITABINE IN PHARMACEUTICAL DOSAGE FORM

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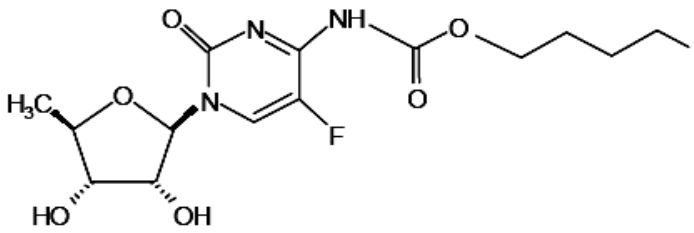
ABSTRACT

A new precise accurate and reliable validated method for the determination of Capecitabine has been developed by using reverse phase high performance liquid chromatography in pharmaceutical dosage forms. Spectrophotometric determination was carried out at an absorption maximum of 230nm by using methanol. The linearity was over the concentration range of 20-120 µg/ml with correlation coefficient 0.999. Chromatographic separation was carried out by using a mobile phase of methanol: Acetonitrile: water (80:18:2 V/V) on Zodiac C18 column (250 mm X 4.6 mm, 5 µm) in an isocratic mode at a flow rate of 1.1 ml/min with UV detection at 230 nm. The developed methods were found to be precise and accurate for the estimation of Capecitabine in pharmaceutical dosage forms and could be used for routine analysis.

Keywords: Capecitabine, RP-HPLC, Spectrophotometry, UV detector,C-18 column,230nm.

INTRODUCTION

Capecitabine is a fluoropyrimidine carbonates with antineoplastic activity and it is in a class drugs known as anti-metabolites. Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [1,2].



5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] - cytidine

Figure 1: Chemical structure of Capecitabine

Capecitabine is a prodrug of 5'-deoxy-5- fluorouridine (5'-DFUR), which is enzymatically converted to 5-fluorouracil in the tumour cells, where it inhibits DNA synthesis and slows growth of tumour 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 [3, 4]. 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. Literature review reveals that few analytical methods have been evoked for the estimation of Capecitabine by HPLC [6-11] method.

EXPERIMENTAL

Instrumental apparatus:

The method development and validation was carried on PEAK LC 7000 with UV Detector and Zodiac, (C18, 250 mm × 4.6 mm × 5µ) as column and Shimadzu, Techcomp-2301 Specophotometer with Hitachi Software was used to determine the wavelength of maximum absorption. In the present work the validation of the method was carried out as per ICH guidelines.

Chemicals and Reagents:

HPLC grade acetonitrile, methanol and water were purchased from Merck Company, Mumbai, India.

Preparation of standard solutions:

About 100 mg of Capecitabine was accurately weighed and transferred into a 100 ml volumetric flask and diluted to volume with methanol to get the stock solution. This gave a concentration of 1000 µg / ml.

Preparation of working stock solutions:

0.6 ml of stock solution was pipetted out and placed in a 10 ml volumetric flask and the volume was made up to mark with methanol. This gave a solution containing 60µg/ml.

Preparation of mobile phase:

A mixture of Methanol, acetonitrile and water (80:18:2 v/v) was employed as a mobile phase. 400 ml of methanol, 90 ml of ACN and 10 ml of water was mixed and sonicated for 15 min. This was filtered by using a 0.45 µm filter paper.

Sample preparation:

Ten tablets were weighed and finely powdered. The powder equivalent to 100 mg of Capecitabine was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the methanol and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. The above solution was filtered through 0.45 µm filter paper. From this solution 0.6 ml was diluted to 10 ml with methanol to give a solution 60 µg/ml.

Determination of λ_{max} by UV spectrophotometer:

The standard solutions of Capecitabine were scanned in the range of 200-400nm against methanol as a blank. Capecitabine showed maximum absorbance at 230 nm. So the wavelength selected for the determination of Capecitabine was 230 nm.

RESULTS AND DISCUSSION

Method development:

HPLC chromatographic separation were carried out in an isocratic mode by using the following optimized conditions were shown in table 1 and the corresponding chromatogram was shown in Figure 2.

Sl. No	Parameter	Result
1	Standard concentration	60 µg/ml
2	Mobile phase	ACN : MeOH: Water (18: 80: 2)
3	Elution	Isocratic
4	Wave length	230
5	Column	Zodiac C_{18} (250mm×4.6mm×5µ)
6	Flow rate	1.1 ml/min
7	Retention time	6.92
8	Run time	10
9	Peak area	198943
10	Theoretical plates	11644.32
11	Tailing factor	1.37
12	Pump pressure	4.8 psi

Table 1: Optimized Chromatographic Conditions

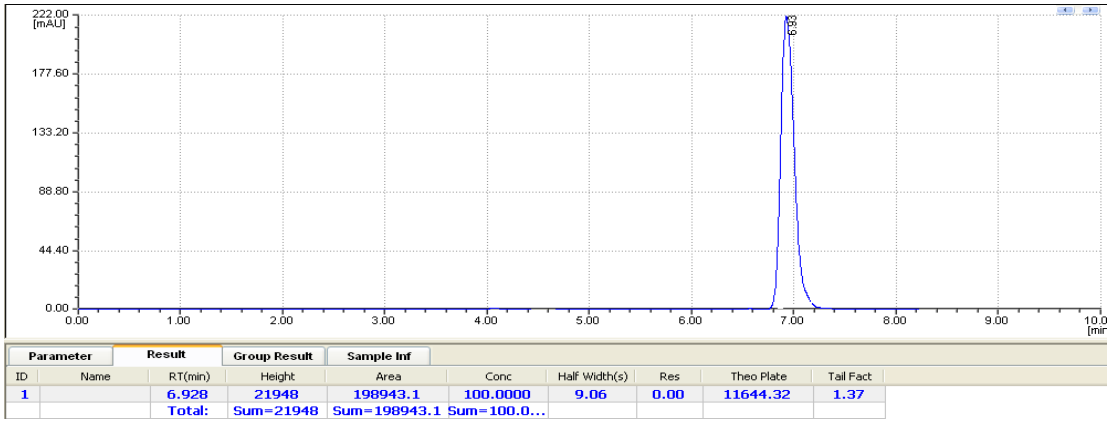


Figure 2: Standard Chromatogram of Capecitabine

Method validation:

Specificity:

The specificity test of the proposed method was demonstrated by blank interference i.e. the blank chromatogram did not interfered with that of the drug peak.

Linearity and range:

Chromatographic method was tested for linearity by plotting peak area against concentration of solution. Linearity ranges and correlation coefficients obtained were presented in Table 2. Linearity plot of

Capecitabine was shown in Figure 3.

Sl. No	Concentration µg/ml	Peak Area
1	0	0
2	20	63585
3	40	144711
4	60	198943
5	80	260546
6	100	334165
7	120	394125
Range 20µg/ml to 120µg/ml	Slope	3284.5
	Intercept	2364
	Correlation coefficient	0.999

Table 2: Results for Linearity

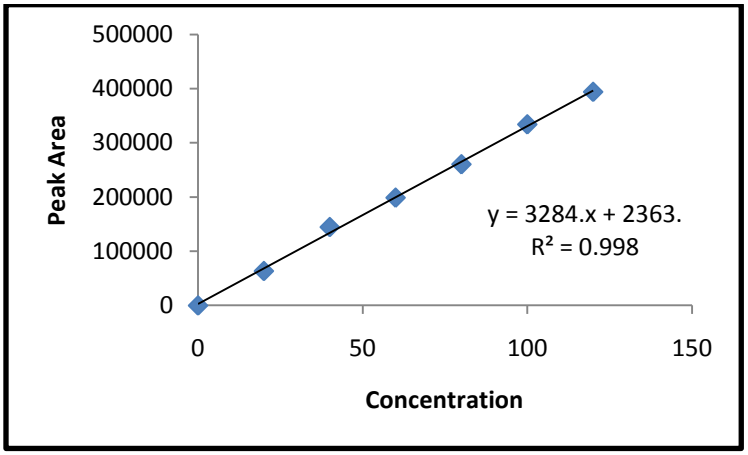


Figure 3: Results of linearity

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-analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting triplicate injections at three different concentrations levels equivalent to 50 %, 100 %, and 150 % 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 98.22-101.49% and were given in table 3.

Concentration level	Capecitabine				
	Target Concentration (µg/ml)	Spiked Concentration (µg/ml)	Final Concentration (µg/ml)	Amount recovered	% Assay
50%	40	20	60	59.36	98.93
	40	20	60	60.24	100.40
	40	20	60	59.38	98.97
100%	40	40	80	81.10	101.38
	40	40	80	78.57	98.22
	40	40	80	81.02	101.27
150%	40	60	100	101.49	101.49
	40	60	100	99.31	99.31
	40	60	100	99.02	99.02

Table3: Results for accuracy

Precision of the method:

The intra-day and inter-day variations of the method were determined by using six replicate injections of one concentration and analysed on the same day and three different days over a period of two weeks.

The result revealed that, the precision with %RSD (0.66% and 1.02%) respectively for intra-day and inter-day. Results of intra-day and inter-day precision studies are shown in table 4A and 4B.

Sample	Conc. in (µg/ml)	Injection No.	Peak Areas	% RSD
Capecitabine	60	1	543590	0.66
		2	543097	
		3	543189	
		4	543765	
		5	543980	
		6	543690	

Table 4A: Results of Intra-day precision

Sample	Conc. in (µg/ml)	Injection No.	Peak Areas	RSD
Capecitabine	60	1	197408	1.027
		2	195435	
		3	197207	
		4	195871	
		5	191836	
		6	195847	

Table 4B: Results of inter-day precision

Limit of detection (LOD) and Limit of quantification:

To determine the Limit of Detection the sample was dissolved by using Mobile Phase and injected until peak was disappeared. After 2µg/ml dilution, Peak was not clearly observed. So it confirms that 0.625 µg/ml is limit of Detection. And Limit of Quantification found to be 2µg/ml. For this study six replicates of the sample at lowest concentration were Measured and quantified. The LOD and LOQ were found to be 0.6µg/ml and 2µg/ml respectively.

Robustness:

The robustness study was performed by slight modification in flow rate and composition of the mobile phase. Capecitabine at 60µg/ml concentration was analyzed under these changed experimental conditions. In this study, the chromatographic parameters monitored were, retention time, area, capacity factor, tailing factor and theoretical plates. The obtained results of robustness study are shown in table5.

Sl. No	Parameter	Condition	Area	% of change
1	Standard	Standard condition	1935218	……..
2	Mobile phases change	MeOH: ACN:WATER 78:20:02	195227	1.86
		MeOH: ACN: Water 82:16:2	201000	1.03
3	Flow change	0.9ml/min	199982	0.52
		1.3ml/min	202667	1.87
4	Wave length change	228nm	201715	1.39
		232nm	197356	0.79

Table5: Results of Robustness

Ruggedness:

Inter day variations were performed by using six replicate injections of standard solution of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample	Conc. (µg/ml)	Injection No.	Peak Areas	% RSD
Capecitabine	60	1	187329	1.33
		2	189703	
		3	187455	
		4	193688	
		5	187765	
		6	191069	

Table6: Results of Ruggedness

System suitability parameters:

The system suitability tests were carried out on freshly prepared standard solution (60µg/ml) of Capecitabine under the optimized chromatographic conditions .from that the parameters that were studied to evaluate the suitability of the system were: a) No. of theoretical plates b) tailing factor c) retention time.

Estimation of Capecitabine in Tablet formulation:

The assays for the marketed tablets were established with present chromatographic conditions and it was found to be more accurate and reliable. The results were shown in table 7.

S.NO	Tablet	Dosage	Sample Conc.	Sample estimated	% of Drug Estimated in Tablet
1	Distamine	100mg	60 µg/ml	98.80 µg/ml	98.80%

Table 7: Formulation analysis results

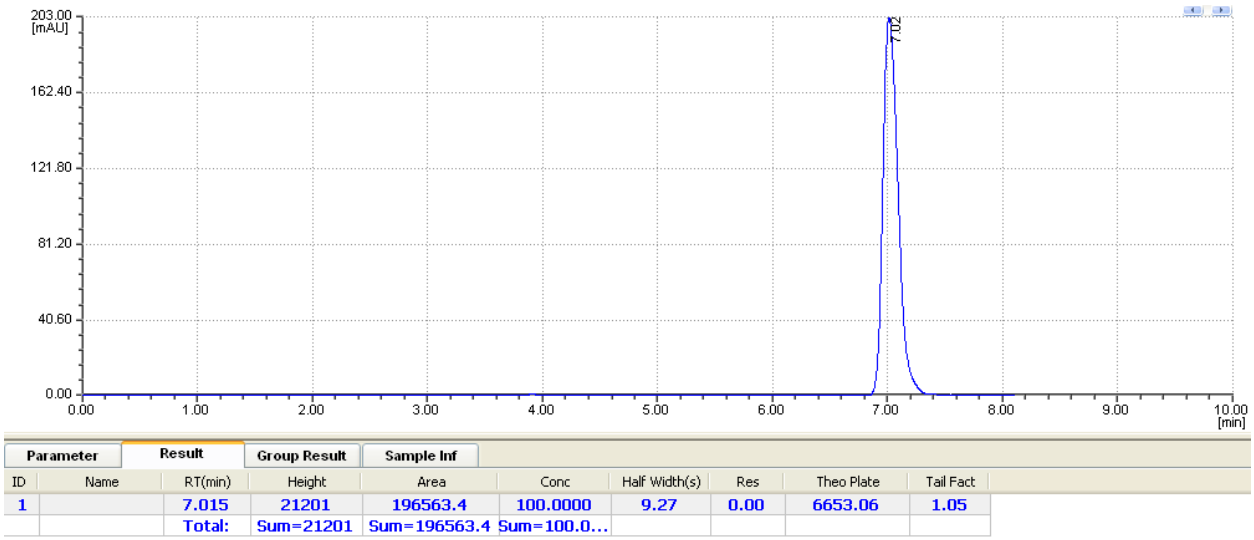


Figure 4: Formulation Chromatogram of Capecitabine

CONCLUSION

The proposed method for the assay of Capecitabinein tablets is very simple, precise accurate and rapid. It should be emphasized that it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ, robustness and system suitability. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. Although the method could effectively separate the drug from its formulation; further studies should be performed in order to use it to evaluate the stability of the formulations.

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