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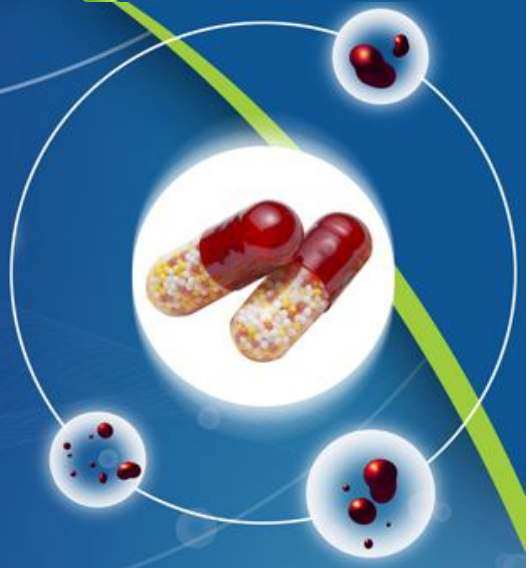

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Research Article

"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CAPECITABINE FROM TABLET DOSAGE FORM BY USING RP-HPLC"

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ABSTRACT

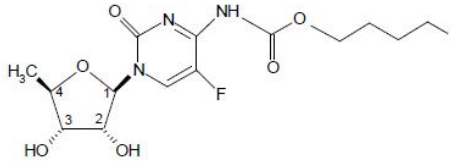
A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Capecitabine in tablet dosage form by using a mobile phase consisting mixture of Methanol: Water in the ratio (85:15 % v/v) at the flow rate of 1 mL/min. Symmetry Chromosil C-18 (4.6×250mm), was used as stationary phase. The UV detection wavelength was 303 nm and 10µl sample was injected. The retention time for Capecitabine was 4.2 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Capecitabine in tablet dosage form.

Key words: *Capecitabine, RP-HPLC Method.*

INTRODUCTION:

Analytical technique play important role in maintaining and assuring the quality of substance and are critical components of quality assurance and quality control. In general terms pharmaceutical analysis comprises, those procedures necessary to determine the identity, strength, quality and purity of drugs and chemicals.

Capecitabine (CAP) [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 tumors (1-3). 5-FU has also been widely used as an anticancer agent in the chemotherapy of solid tumors but its efficacy is limited by dihydropyrimidine dehydrogenase catalyzed formation of dihydro-5-fluorouracil. Since it lacks selectivity toward tumor cells, 5-FU also exhibits significant toxicity. Prodrug of 5-FU have been developed to improve efficacy and to reduce side effect and toxicity (4-5). For example, Tegafur [5-fluoro-1-[(RS)-tetrahydrofuran-2-yl]-pyrimidine-2, 4-(1H, 3H)-Dione] maintains an effective 5-FU concentration over a longer period while Doxifluridine [5_deoxy-5-fluorouridine] achieves some selectivity toward tumors (6-7). However, both prodrugs still show adverse effects, such as diarrhoea after oral and intravenous administration. CAP was developed to reduce such adverse effects while improving the selectivity toward tumours. (8)



Structure of Capecitabine

The aim of present work is to find out a simple, sensitive, specific& accurate method developed

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for the detection of CAP in bulk drug and pharmaceutical formulation.

Hence, on the basis of literature survey it was thought to develop a precise, accurate, simple, reliable and less time consuming RP –HPLC method for estimation of Capecitabine in tablet formulations.

MATERIALS & METHODS:

Apparatus:

The analysis was performed by using Waters HPLC system 400, Column used is C-18 (4.6×250mm) with a flow rate of 1 ml/min. The mobile phase consists of Methanol: Water as 85:15, the injection volume is 10µL and the photo diode array detection was at 303 nm.

Reagents and materials:

CAP was obtained as a gift sample from Naprod Life Sciences, Boisar India. All solvents and reagents used were of AR/HPLC grade. A tablet dosage form containing CAP were purchased from local commercial sources.

Standard solution-

Selection of Mobile Phase:

Selection of mobile phase was performed based on resolution, asymmetric factors and theoretical plates obtained for the drug.

By consideration of pKa values of the drugs, initially pH was adjusted to both acidic and basic pH range.

From the various mobile phases tried, mobile phase containing methanol: Double distilled water in 85:10 % v/v, pH was adjusted to7.5 with NaOH was selected, since it gave sharp and completely resolved peaks with symmetry within limits and significant retention times for the drug.

Preparation of standard drug solution:

Accurately weighed quantity 500mg of Capecitabine was transferred to 1000.0 ml volumetric flask, dissolved the mixture using 100 ml of distilled water as a diluent and then sonicated for 10 minutes. Later the volume was made up to the mark with diluent to make 500 ppm of Capecitabine.

Preparation of sample solution:

Marketed tablet formulation containing 500 mg CAP of Capecitabine was used for preparation of sample solution.

Twenty tablets were weighed accurately, finely powdered and powder equivalent to 500 mg of Capecitabine was transferred into 100 ml volumetric flask, dissolved the mixture in 25 ml of diluent and sonicated for 10-15 minutes. The final volume of the solution was made up to 100 ml with diluent, and the solution was filtered through whatmann filter paper no.41.The sample was analyzed under optimized chromatographic conditions and chromatogram is depicted in Figure 4.The result of analysis of marketed tablet formulation shown in Table 3.And 4

The final dilutions of standard and sample were injected to the system and analyzed at 303 nm. Capecitabine was eluted at the retention time of 4.2 min. for Capecitabine

Analytical Method Validation: [1-8]

The method was validated in terms of linearity, sensitivity, precision and accuracy the sample applications. The linearity of the method was investigated by serially diluting the stock solutions. Calibration curves were constructed by plotting the peak area against the concentration. All the drugs show linearity in the concentration range. Method was validated as per ICH Q2B guidelines.

Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Capecitabine was found to be 0.5-1.5 µg/ml.

Accuracy:

Accuracy of an analytical method is the closeness of test results obtained by a method to the true value. The accuracy of analytical method should be established across its range. To check the degree of accuracy of the proposed method, recovery studies were carried at three different levels (80%, 100% and 120%).From the total amount of drug added, the percentage recovery was calculated [1,2,7,8].

Preparation Chart for Accuracy solution is shown in Table 4

Solution was subjected to the proposed HPLC method. Chromatograms were

represented in Figure 5.to 7

Precision:

The precision of an analytical method is the amount of scatter in the results obtained from multiple analyses of a homogeneous sample under the prescribed conditions.

Precision (Intra-day precision) was evaluated by carrying out replicate injections of CAP sample preparation on the same day [1-2]. The results were shown in Table 5.and 6.

Robustness:

Robustness is the measure of its capacity to remain unaffected by small, but deliberate variations in method conditions and its indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of method, the experimental conditions were deliberately altered using following parameters and chromatographic responses were evaluated [1,4-8].

1) Change in flow (± 0.1 ml/min)
2) Change in wavelength (± 2 nm)

Standard and test solutions for each of the robustness parameter were prepared and injected in the system. Their effects on retention time, theoretical plate, tailing factor, percent recovery, %R.S.D. were standard. The results from the robustness studies are presented in Table 7 & 8 and Figure 8 &9

Specificity:

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components

The response of the analyte in test mixtures containing the analyte and all potential sample components (excipients, degradation products, process impurities, etc.) is compared with the response of a solution containing only the analyte [1, 7].

The peak purity of Capecitabine was assessed by comparing the retention time (Rt) of standard Capecitabine.

System Suitability: [1-2, 9, 13]

System suitability tests are an integral part of method development is used to ensure adequate performance of the chromatographic system. Retention time, No. of theoretical plates (N), tailing factor (T), and peak symmetry (AS) were recorded. The results were given in Table 10 and are within acceptable limits.

RESULT AND DISCUSSION:

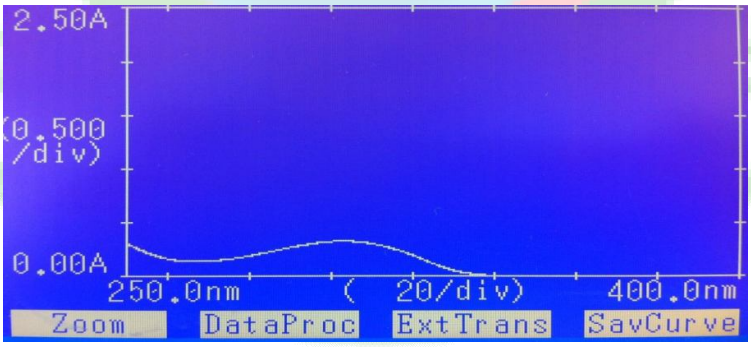

Figure.1: Spectra UV Scan of Capecitabine (CAP) in distilled water.

Table No1: Data for calibration curve of CAP

Sr. No.	Conc.(µg/ml)	Peak area
1.	50	219489
2.	75	447109
3.	100	659469
4.	125	881887
5.	150	1082382

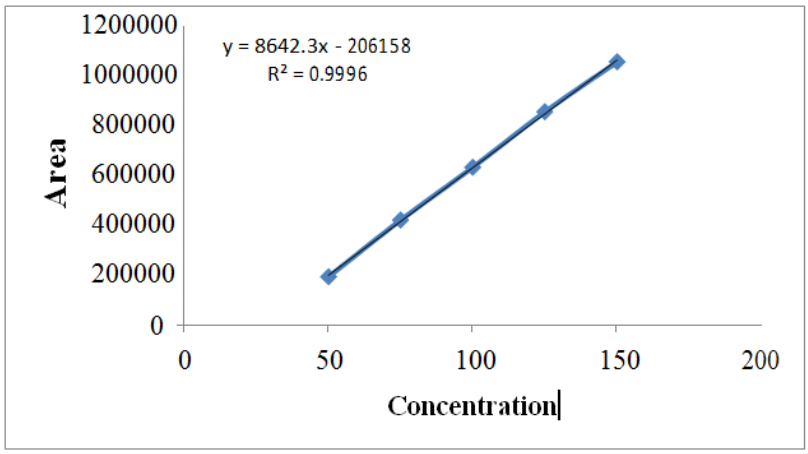

Table No.2: Statistical data of linearity for CAP by HPLC method

Sr. No.	Parameters	HPLC method
1.	Detection wavelength(nm)	303nm
2.	Beer's law limit(µg/ml)	50-150
3.	Regression equation Y*	8642.3x - 206158
4.	Slope (B)	8642.3
5.	Intercept(A)	206158
6.	Correlation coefficient r2	0.999

Y= A + B*C, where C= concentration in µg/ml and Y =absorbance unit.

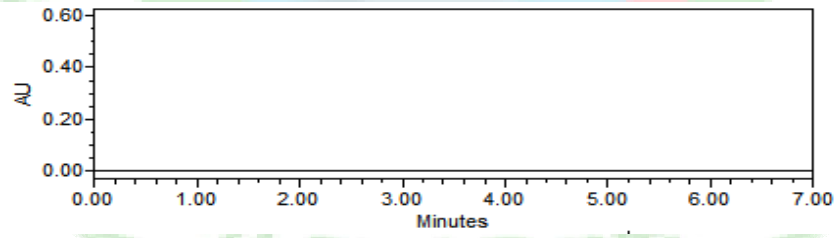

Figure 2: Chromatogram of Blank

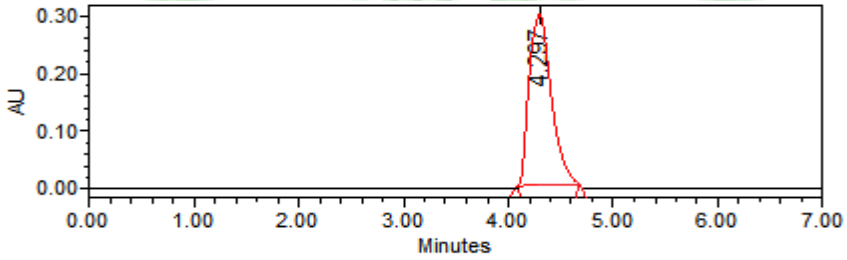

Figure 3: Chromatogram of standard

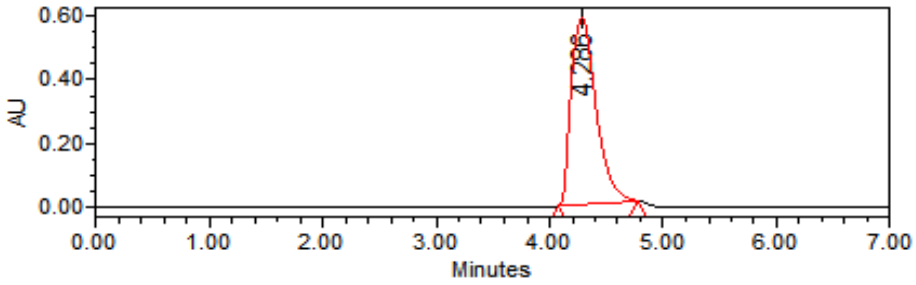

Figure 4: Chromatogram of sample

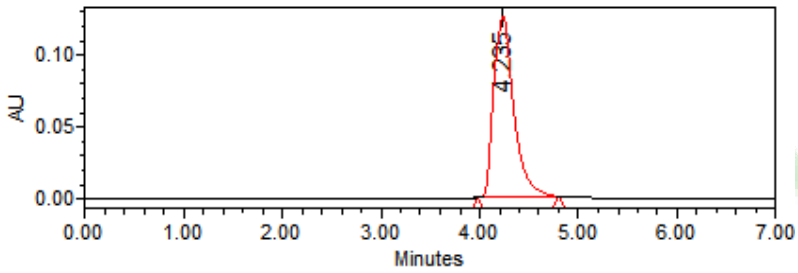

Figure 5: Chromatogram of accuracy level I (80%)

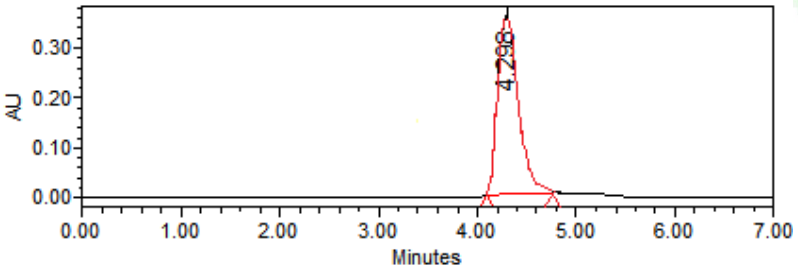

Figure 6: Chromatogram of accuracy level II (100%)

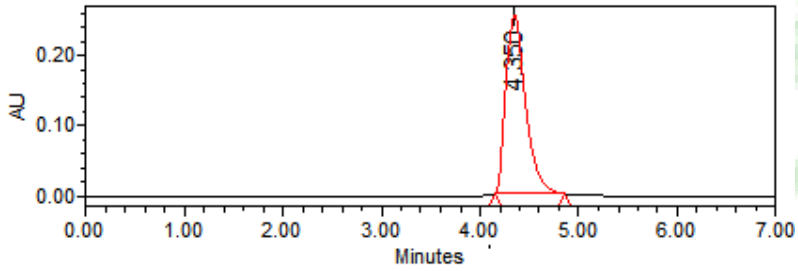

Figure 7: Chromatogram of accuracy level III (120%)

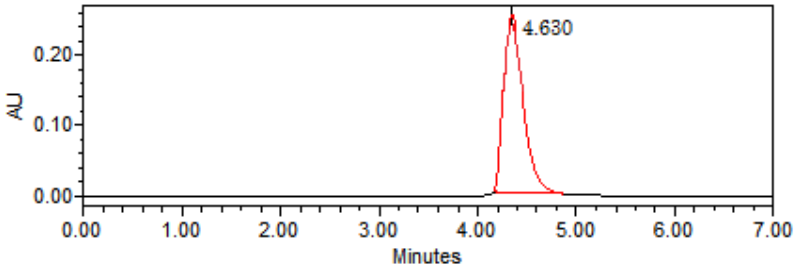

Figure 8: Chromatogram of standard preparation (0.9ml/min flow rate)

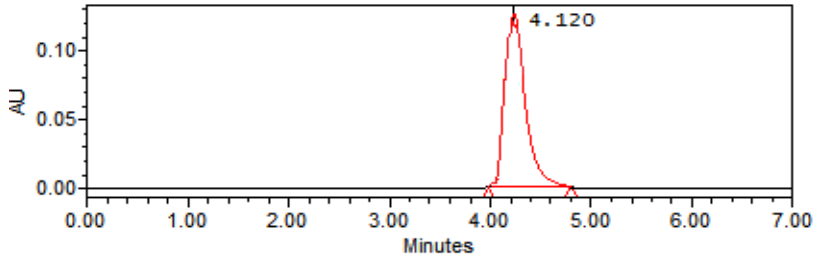

Figure: 9 Chromatogram of standard preparation (1.1ml/min flow rate)

Table No 3: Results of Analysis of Marketed Formulation

Drug	Label Claim	Amount Found	% RSD	% Recovery
CAP	500	498.5	0.765	99.7

Table No 4: Result of Accuracy

Recovery Level	Total Amount (mg)	Amount Found (mg)	% Recovery	Mean % Recovery SD	% RSD
80%	179.82	179.71	99.86	99.92±0.11	0.1123
	179.76	179.64	99.84		
	179.92	179.96	100.05		
100%	199.91	199.82	99.90	99.00±0.02	0.0200
	199.87	199.76	99.88		
	199.89	199.82	99.92		
120%	219.84	219.75	99.92	99.98±0.10	0.1059
	219.90	219.81	99.92		
	219.83	219.96	100.10		

Table No.5: Results of Intra-Day Precision

Time	Label Claim	% Label Claim estimated (Mean±S.D.)	%RSD
Morning	500	99.80±0.69	0.6929
Afternoon		100.12±71	0.7285
Evening		99.96± 0.69	0.6986

Average of six determinations; %R.S.D. Relative standard deviation

Table No.6: Results of Inter-Day Precision

Time	Label Claim	% Label Claim estimated (Mean±S.D.)	%RSD
Day 1	500	99.45±0.55	0.5478
Day 2		99.80±54	0.5580
Day 3		100.04± 0.63	0.6567

Average of six determinations; %R.S.D. Relative standard deviation

Table No 7: Results of Ruggedness

Analyte		Label Claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	% R.S.D.
Analyst I	CAP	500	99.34±0.115	0.116
Analyst II			100.21 ± 0.129	0.134

Table No.8: Results of Robustness

Sr. No	Parameter	Optimized	Used	Retention time (tR),Min	Plate count$
1	Flow rate (±0.2mL/min)	1.0mL/m in	0.9mL/m in	4.630	10426
			1.1mL/m in	4.130	9724
2	Detection wavelength(±2nm)	303nm	301nm	4.282	10080
			305nm	4.329	10032
3	Mobile phase composition (±5%)	85:15 v/v	+5%	4.354	10360
			-5%	3.916	9856

Table No.9: Result of LOD & LOQ

LOD (µg/ml)*	LOQ (µg/ml)*
0.12	0.59

Table No10: System Suitability Test Parameters

Parameter	CAP
Calibration range (µg/ml)	50-150µg/ml
Theoretical Plates	5374.43
Resolution	2.008
Tailing Factor	1.3714
Asymmetry	1.5398
Slope	8642.3
Intercept	2061
Correlation Coefficient(r^2)	0.9996
Retention time (min.)	4.297

CONCLUSION:

The proposed method was validated in accordance with ICH Q2R guidelines. The calibration was linear in concentration range of 0.5-1.5 µg/ml for CAP with correlation coefficient of 0.999.The accuracy of method was indicated by recovery values of 99.99 to 100.00%. Precision is reflected by % RSD values less than 2.0.Limit of detection and Limit of quantitation were determined by standard deviation of response and slope of calibration curve. The %LOD CAP was found to be 0.12µg/ml for CAP. The % LOQ for CAP was found to be 0.59µg/ml.

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