A NOVEL METHOD DEVELOPMENT AND VALIDATION FOR CHROMATOGRAPHIC DETERMINATION OF CAPECITABINE HCL IN PURE FORM AND FORMULATION BY USING RP-HPLC

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Abstract : The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Capecitabine in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Symmetry C18, 250 mm x 4.6 mm and 5µm Column, Mobile Phase: 0.01M Phosphate Buffer(P^H 5.2): Acetonitrile = 45 : 65, flow 1.0 ml/min with Injection Volume 20µl, at detection wavelength 243 nm, run time at 06mins and Retention time was 4.398mins.The analytical method is valid for estimation of Capecitabine over a range of 0-35 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Capecitabine has been developed based on ICH Guidelines with bulk and dosage forms.

Key Words: Capecitabine, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

I.INTRODUCTION

Capecitabine is a prodrug that is selectively tumour-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase, an enzyme found in higher concentrations in many tumors compared to normal tissues or plasma. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumour cells. These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N5-10-methylenetetrahydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex.

IUPAC Name : pentyl N-{1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl}carbamate

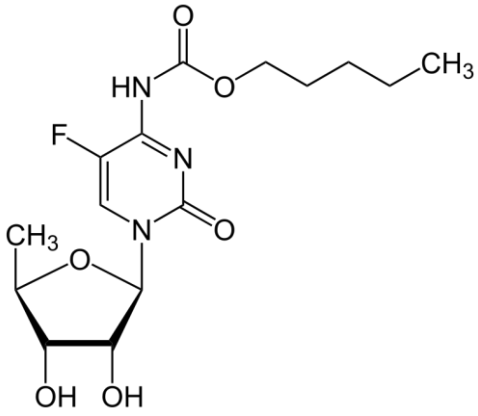


Fig-1: Structure of Capecitabine

II.EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Capecitabine were obtained from Orchid Pharma from India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (T60-LAB INDIA), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry C18 Column, 250 mm x 4.6 mm and 5µm (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

10 mg of Capecitabine standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 100ppm concentration. Then, the final concentration was made by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It is scanned in the UV spectrum in the range of 200 to 400nm.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in methanol diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Capecitabine solution we observed the maxima at 243 nm.

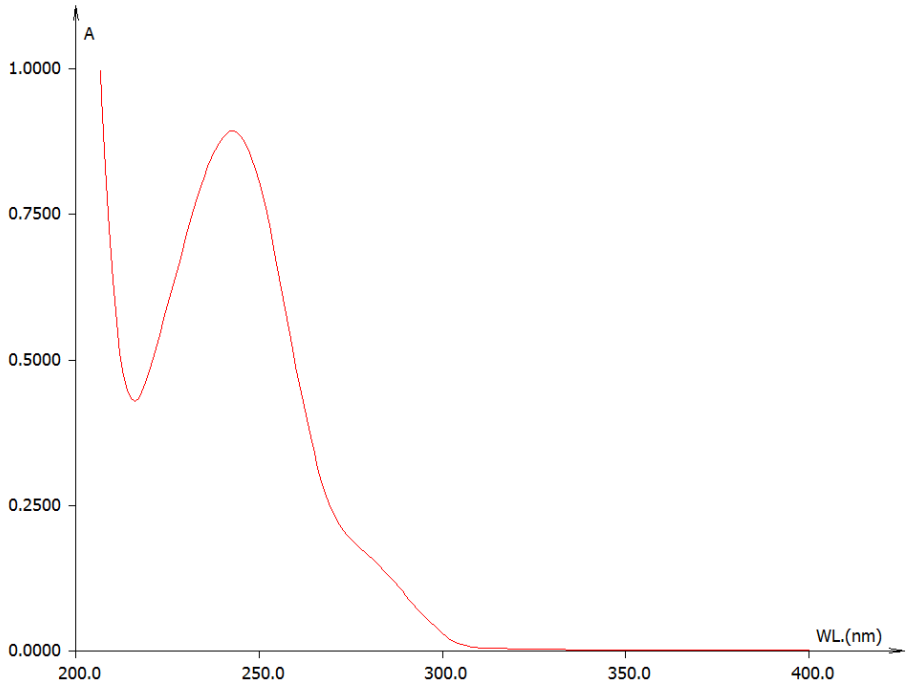


Fig-2: UV Spectrum for Capecitabine

2.5 Method Development

2.5.1 Preparation of Mobile Phase:

450ml of Phosphate buffer (pH 5.2) and 650ml of HPLC Grade Acetone were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.5.2 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimized Chromatographic Conditions

Mobile phase	0.01M Phosphate Buffer(P^H 5.2): Acetonitrile = 45 : 65
Column	Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm
Column Temperature	Ambient

Detection Wavelength	243 nm
Flow rate	1.0 ml/ min.
Run time	09 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase

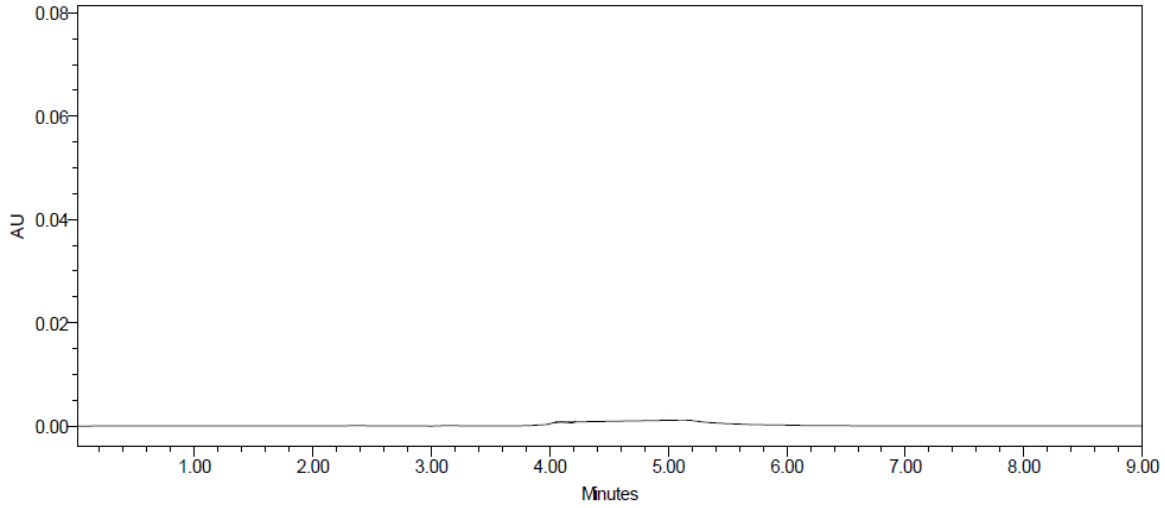
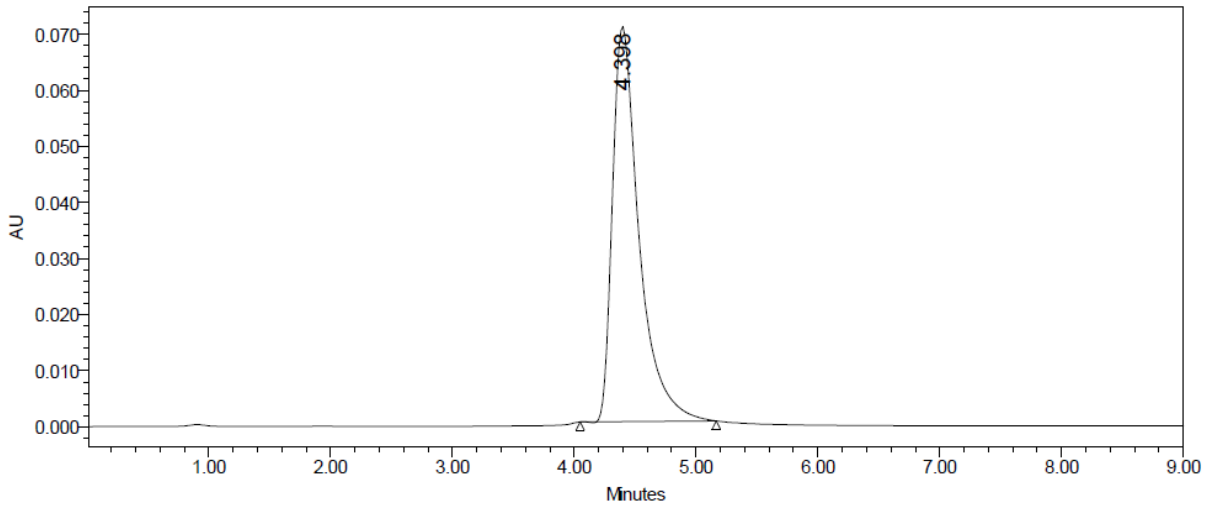


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Capecitabine in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 0-70µg/ml for Capecitabine. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

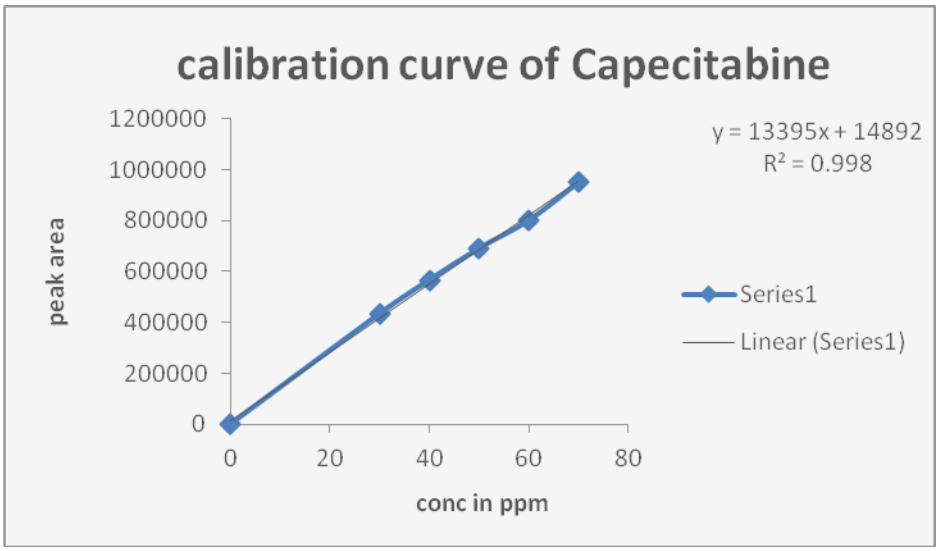


Fig-5: Calibration Curve of Capecitabine (API)

Table-2: Concentration of Capecitabine

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	30	433629
3	40	565842
4	50	689871
5	60	799563
6	70	949312

2.6.2. Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Capecitabine were taken and added to the pre-analyzed formulation of concentration 50µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	39.43539	528252	98.58849	Mean= 99.47315% S.D. = 1.102797 % R.S.D.=1.108637
S_2 : 80 %	40	40.28347	539612	100.7087	
S_3 : 80 %	40	39.64891	531112	99.12227	
S_4 : 100 %	50	49.34827	661035	98.69654	Mean= 99.62055% S.D. = 0.183955 % R.S.D.= 0.1865280
S_5 : 100 %	50	49.20538	659121	98.41077	
S_6 : 100 %	50	49.37716	661422	98.75433	

S_7 : 120 %	60	59.59732	798321	99.32887	Mean= 99.87567% S.D. = 0.775816 % R.S.D.= 0.7767817
S_8 : 120 %	60	59.72072	799974	99.53454	
S_9 : 120 %	60	60.45816	809852	100.7636	

2.6.3. Precision:

2.6.3.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Capecitabine (API). The percent relative standard deviation was calculated for Capecitabine are presented in the Table-4.

Table-4: Repeatability Readings of Capecitabine

HPLC Injection Replicates of Capecitabine	**Peak Area**
Replicate – 1	9677961
Replicate – 2	9739160
Replicate – 3	9673810
Replicate – 4	9649981
Replicate – 5	9606331
Replicate – 6	9616451
Average	9660616
Standard Deviation	48279.61
% RSD	**0.49975**

2.6.3.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Capecitabine revealed that the proposed method is precise.

Table-5: Results of Intra-Assay & inter-assay

Conc. Of Capecitabine (API) (µg/ml)	Observed Conc. Of Capecitabine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
40	49.98	0.99	41.99	1.05
50	59.99	0.98	59.99	0.96
60	69.98	1.02	68.98	0.95

2.6.4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-6, % RSD < 2%) the developed RP-HPLC method for the analysis of

Capecitabine (API).

Table-6: Results of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.08
Flow (0.9 ml/min)	0.67
Temperature (27^0C)	0.75
Temperature (23^0C)	0.58
Wavelength of Detection (245 nm)	0.63
Wavelength of detection (241 nm)	0.82

2.6.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 0.33(SD/S).

L.O.Q. = 0.10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.003 & 0.009 µg/ml respectively.

2.6.6. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S. No	Parameter	Capecitabine
1	Retention time	Above 2mins
2	Tailing factor	$T \leq 2$
3	Theoretical plate	$N > 2000$

2.6.7 Estimation of Capecitabine in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with mobile phase. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

Table-8: Assay of Capecitabine Tablets

Brand name of Capecitabine	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Xeloda tab	500mg	499.98 (± 0.03)	98.86 (± 0.79)

Result & Discussion: The amount of drug in Xeloda tablets was found to be 499.98 (±0.03) mg/tab for Capecitabine and 99.86 (±0.79) mg/tab for Capecitabine.

III. RESULTS

The optimized The chromatographic conditions were performed on Symmetry C18, 250 mm x 4.6 mm and 5µm Column, Mobile Phase : 0.01M Phosphate Buffer(P^H 5.2): Acetonitrile = 45 : 65 flow 1.0 ml/min with Injection Volume 20µl, at detection wavelength 243 nm, run time at 09 mins and Retention time was 4.398mins.

The results obtained in method validation were :

Linearity & Range: The calibration curve showed good linearity in the range of 0-70µg/ml, for Capecitabine (API) with correlation coefficient (r^2) of 0.998 (Fig-28). A typical calibration curve has the regression equation of y = 13395x - 14892 for Capecitabine..

Accuracy: From the Accuracy Method, we observed that the mean %Recovery of the drug are 100.07 which is within the range of 98-102% and %RSD is within the range <2 i.e. 1.108637%, 0.1865280%% and 0.7767817% respectively

Repeatability: From the Precision method, we observed that the %RSD 0.49975 which are within the acceptable range as per ICH guidelines.

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.003 & 0.009 µg/ml respectively..

Assay: The amount of drug in Xeloda tablets was found to be 499.98 (±0.03) mg/tab for Capecitabine and 99.86 (±0.79) mg/tab for Capecitabine.

4. DISCUSSION: To develop a precise, linear, specific RP-HPLC method for analysis of Capecitabine, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Pani Kumar AD et al, A simple, rapid accurate and stability indicating RP-HPLC method was developed for the determination of Capecitabine in pure and tablet dosage form. The method showed a linear response for concentrations in the range of 70-120 µg/ml using 0.05M phosphate buffer (p H 3.0 ± 0.05) buffer and acetonitrile (50:50 % w/v) as the mobile phase with detection at 240 nm and a flow rate of 1 mL/min and retention time 4.108 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity.

IV. CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Capecitabine API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Capecitabine indicated that the developed method is specific for the estimation of Capecitabine. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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