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RP-HPLC method development and validation of capecitabine in pharmaceutical dosage form

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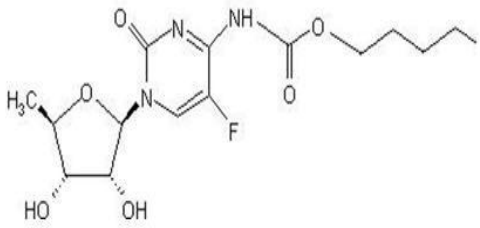
ABSTRACT

A simple, accurate, rapid, and stability-indicating RP-HPLC method for a Capacetabine has been developed and subsequently validated in commercial tablets. The proposed HPLC method utilizes Shimadju ODS (C_8) RP Column, 150 mm x 4.6 mm. and mobile phase consisting of a mixture of Buffer (0.01 M potassium dihydrogen phosphate & pH adjusted to 2.2 with ortho phosphoric acid) and Methanol in a ratio of 40:60 Quantitations was achieved with UV detection at 260 nm. The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation, and robustness. This optimized method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. Capecitabine drug products were subjected to acid, base, neutral hydrolysis, oxidation, dry heat, and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed RP-HPLC method could effectively separate the drugs from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and pharmaceutical dosage form.

Keywords: HPLC, Method development, Validation, Reverse Phase, Capecitabine.

INTRODUCTION

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [1,2]. Capecitabine is a prodrug that is enzymatically converted to fluorouracil (antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue.



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 C15H22FN3O6 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 [5-11] method.

The aim of the study is method development and validation of Capecitabine by RP-HPLC was not accurate showing deviations from system suitability parameters. It was found that previous methods developed showed increased retention time and improper stability related values by using RP-HPLC**.** Hence the objective of the present work is to develop a new precise method and validation of parameters including its stability related impurities. This new method was successfully developed and validated as per ICH guidelines [12-13], can be utilized for the validation of Capecitabine in pharmaceutical dosage forms.

MATERIALS AND METHODS

Table 2.1: List of equipments

Sl	Instruments/Equipments/Apparatus
1.	**Hitachi L2130** with D Elite 6000 Software with Isocratic with UV-Visible Detector (L-2100),
2	**ELICO SL-159** UV – Vis spectrophotometer
2	Electronic Balance
3.	Ultra Sonicator **(Wensar wuc-2L)**
4.	Thermal Oven
5.	**Shimadju ODS (C_8) RP Column, 150 mm x 4.6 mm.**
6.	P^HAnalyzer **(ELICO)**

Table 2.2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	HPLC grade water	----	----	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Ortho phosphoric acid	99.9	L.R.	Sd fine-Chem ltd; Mumbai

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Capecitabine, so that the same wave number can be utilized in HPLC UV detector for estimating the Capacetabine. While scanning the Capacetabine solution we observed the maxima at 242.5 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – VIS spectophotometer model UV-2450. The scanned UV spectrum is attached in the following page

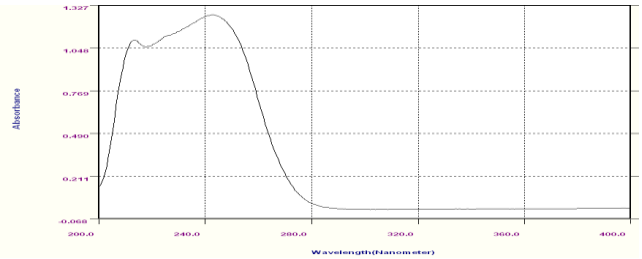


Fig- 2.1.1: UV-Spectrum for Capacetabine

Mobile phase preparation

The mobile phase used in this analysis consists of a mixture of Buffer (0.01 M potassium dihydrogen phosphate & pH adjusted to 2.2 with ortho phosphoric acid) and Methanol in a ratio of 40:60

Preparation of Standard solution

Working concentration should be around 40 µg/ml. Accurately weighed around 25mg of Capacetabine working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml. Further dilutions has been made to get the final concentration of 40 µg/ml

Preparation of Test solution

Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range.

DIFFERENT TRIALS FOR CHROMATOGRAPHIC CONDITIONS

Different chromatographic conditions

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol : Water = 80 : 20	0.8ml/min	242.5 Nm	Very Low response	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN : Water = 80 : 20	0.5ml/min	242.5 Nm	Tailing peak	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN: water = 70 : 30	1.0ml/min	242.5 Nm	Peak Broken	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol : acetate buffer = 75 : 25	1.0ml/ min	242.5 Nm	Extra Peaks are there	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol:SPhosphate buffer = 60:40	1.0ml/min	242.5 Nm	Good sharp peak	Method accepted

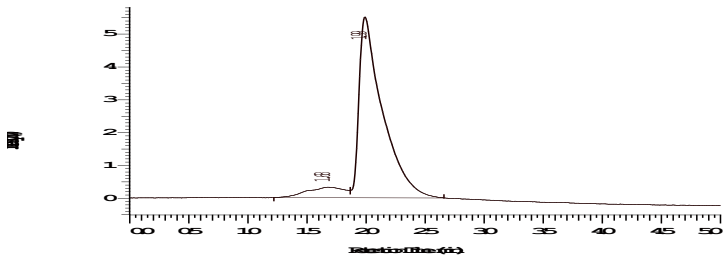


Fig- 2.5.1: Chromatogram for Trial-1

Table2.5.1: Results of Trial-1

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	1.41	7334	2.02	1741

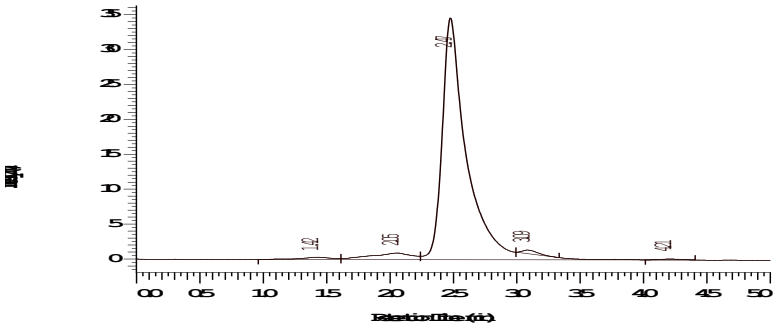


Fig2.5.2: Chromatogram for Trial-2

Table 2.5.2: Results of Trial-2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.47	12334	2.14	1541

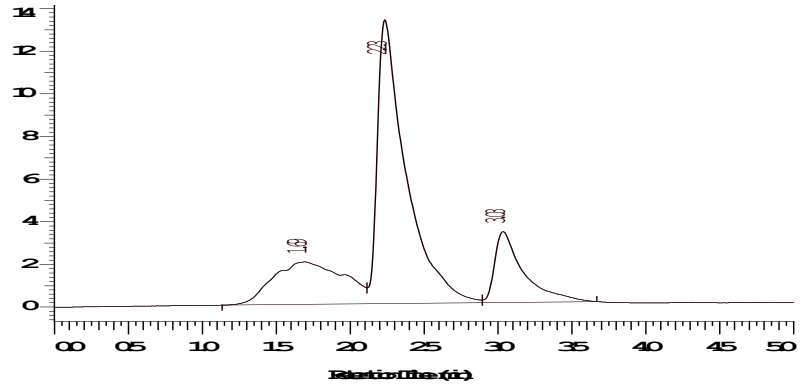


Fig 2.5.3: Chromatogram for Trial-3

Table 2.5.32: Results of Trial-3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.23	12021	2.16	1524

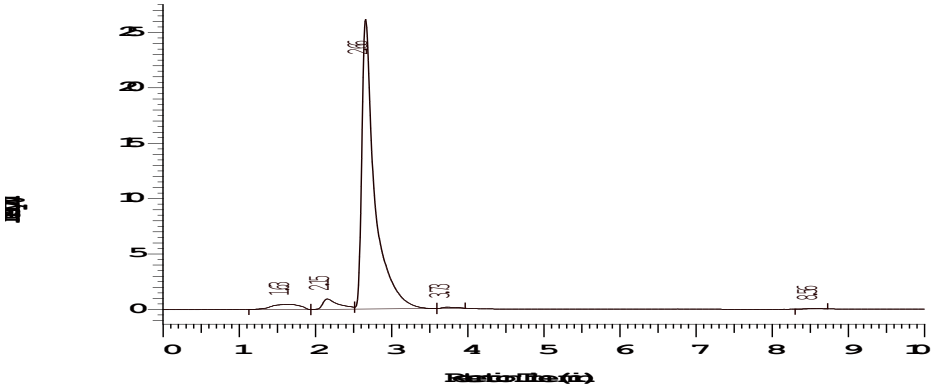


Fig2.5.4: Chromatogram for Trial-4

Table 2.5.4: Results of Trial-4

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.68	11458	1.16	2524

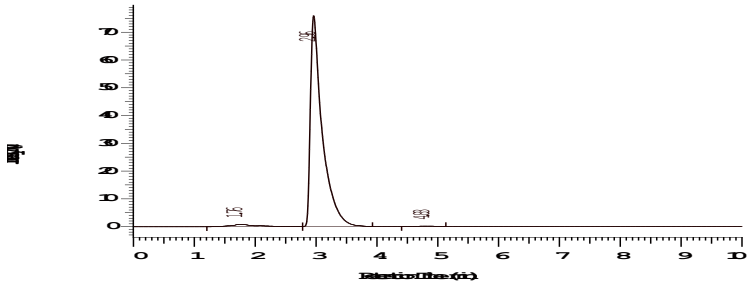


Fig2.5.5: Chromatogram for Trial-5

Table2.5.5: Results of Trial-5

S.NO	RT(min)	PEAK AREA	PEAK CONCENTRATION
1	2.96	1246755	99.89

Optimized Chromatographic Conditions

Column : Waters C_{18}, 5µm, 25cmx4.6mm i.d.
Mobile Phase : Buffer: Methanol (40:60)
Flow Rate : 1.0ml/minute
Wave length : 242.5 nm
Injection volume : 20 µl
Run time : 10 minutes
Column temperature : Ambient

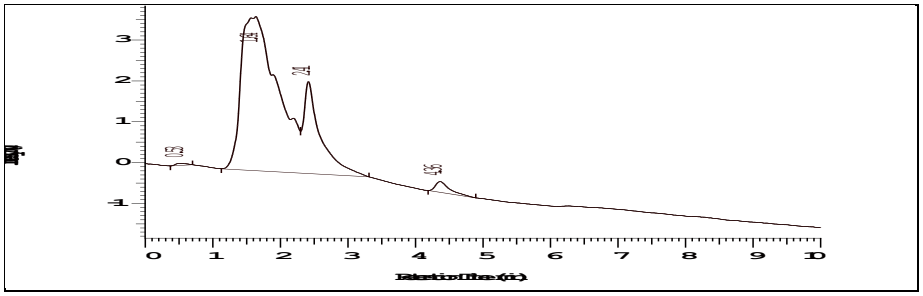


Fig2.6.1: Chromatogram for Blank

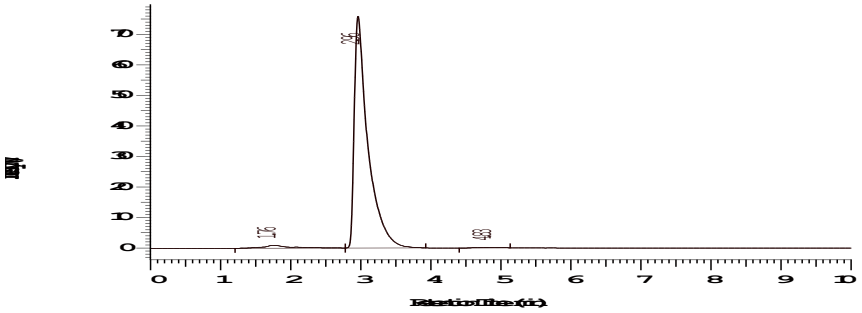


Fig. 2.6.2 Chromatogram for Capacetabine (Rt 2.96 min)

Table 2.6.1: Results of Optimized Condition

S.NO	RT(min)	PEAK AREA	PEAK CONCENTRATION
1	2.96	1246755	99.89

PREPARATION OF MOBILE PHASE AND DILUENT [9-12]

Preparation of Phosphate buffer

6.8 grams of Potassium dihydrogen orthophosphate was Weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. pH was adjusted to 2.2 with Orthophosphoric acid.

Preparation of mobile phase

A mixture of above buffer 400mL (40%) and 600 mL of methanol HPLC (60%) were mixed and degassed in ultrasonic water bath for 15 minutes and filtered through 0.45 µm filter under vacuum filtration.

PREPARATION OF SOLUTIONS FOR STABILITY STUDY IN ACIDIC CONDITIONS & THEIR STORAGE

Preparation of 1N Hcl

8.5ml of Hcl was dissolved in 100ml of HPLC Grade water which gives 1N of 100ml Hcl solution.

Storage of Drug in 1N Hcl

50ml of 1N Hcl solution from above prepared solution was separated and taken into another 50ml volumetric flask in which 50mg of Capacetabine was previously taken. Drug was dissolved by initially adding 30ml of 1N Hcl and shaking it. Then remaining amount of 1N Hcl was added upto the mark to prepare 50ml of 1N Hcl solution with 50mg of Drug dissolved in it which constitutes 1000ppm solution. This solution was stored for 24hrs to allow the degradation of drug.

PREPARATION OF SOLUTIONS FOR STABILITY STUDY IN ALKALINE CONDITIONS & THEIR STORAGE:

Preparation of 1N NaOH

4gm of sodium Hydroxide was dissolved in 100ml of HPLC Grade water to prepare 1N NaOH solution.

Storage of Drug in 1N NaOH

50ml of 1N NaOH solution from above prepared solution was separated and taken into another 50ml volumetric flask in which 50mg of Capacetabine was previously taken. Drug was dissolved by initially adding 30ml of 1N NaOH and shaking it. Then remaining amount of 1N NaOH was added upto the mark to prepare 50ml of 1N NaOH solution with 50mg of Drug dissolved in it which constitutes 1000ppm solution. Then it was sonicated for about 15mins to dissolve the drug completely. This solution was stored for 24hrs to allow the degradation of drug.

PREPARATION OF SOLUTIONS FOR STABILITY STUDY IN OXIDATIVE CONDITIONS & THEIR STORAGE

Preparation of 1N H_2O_2

2.9ml of H_2O_2 was dissolved in 100ml of HPLC Grade water to prepare 100ml of 1N H_2O_2 solution.

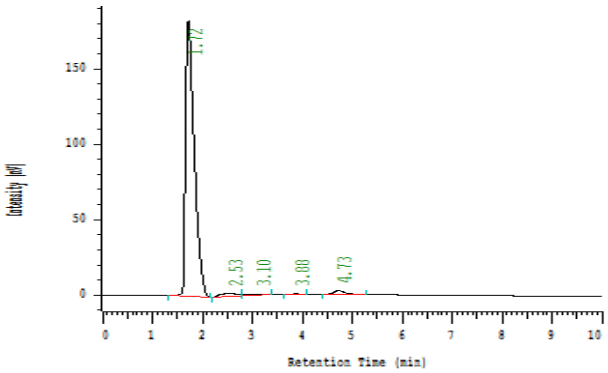
Storage of Drug in 1N H_2O_2

50ml of 1N H_2O_2 solution from above prepared solution was separated and taken into another 50ml volumetric flask in which 50mg of Capacetabine was previously taken. Drug was dissolved by initially adding 30ml of 1N H_2O_2 and shaking it. Then remaining amount of 1N H_2O_2 was added upto the mark to prepare 50ml of 1N H_2O_2 solution with 50mg of Drug dissolved in it which constitutes 1000ppm solution. This solution was stored for 24hrs to allow the degradation of drug.

STABILITY STUDY OF CAPECITABINE IN ACIDIC CONDITION (1N HCL)

Procedure

Prepare working standard of 40ppm solution from stored 1000ppm 1N Hcl solution with drug dissolved in it by taking 1ml of it into 10ml volumetric flask and adding diluents to it upto mark which gives 100ppm solution and again repeating same procedure by taking 1ml of 100ppm solution to finally prepare 10ppm solution. Inject blank 1N Hcl into HPLC and run it. Then inject 10ppm solution prepared in 1st step into HPLC and run it.



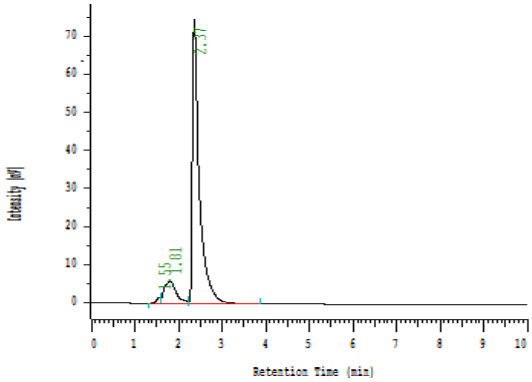


Fig2.10.1: Chromatogram of 1N Blank Hcl: **Fig2.10.2:** Chromatogram of stability study of Drug in 1N Hcl:

Table 2.10.1: Results of Acid hydrolysis

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.37	546104	2.16	1524

STABILITY STUDY OF CAPECITABINE IN ALKALINE CONDITION (1N NAOH) :

Procedure

Prepare working standard of 40ppm solution from stored 1000ppm 1N NaOH solution with drug dissolved in it by taking 1ml of it into 10ml volumetric flask and adding diluents to it upto mark which gives 100ppm solution and again repeating same procedure by taking 1ml of 100ppm solution to finally prepare 10ppm solution[13]. Inject blank 1N NaOH into HPLC and run it. Then inject 10ppm solution prepared in 1st step into HPLC and run it.

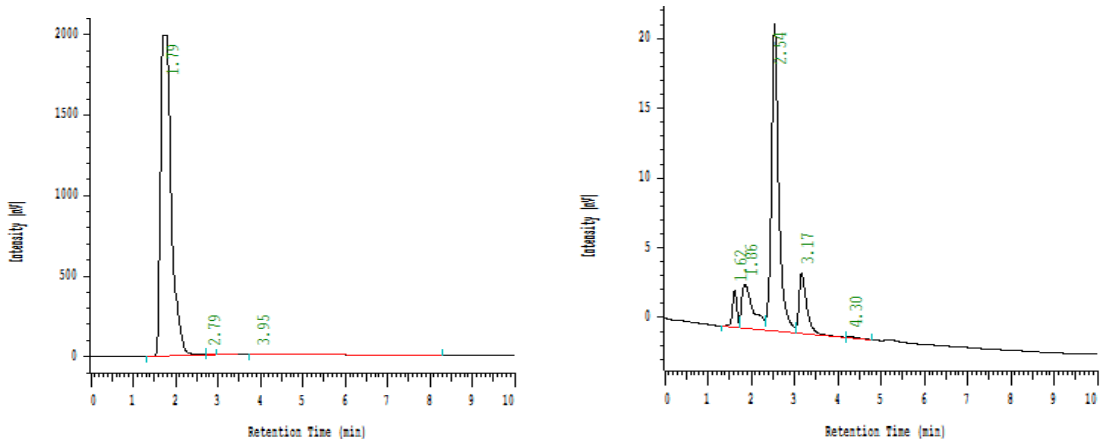


Fig 2.11.1: Chromatogram of 1N Blank NaOH: & Chromatogram of stability study of Drug in 1N NaOH

Table 2.11.2: Results of Basic hydrolysis

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPACETABINE	2.54	587104	2.16	1524

STABILITY STUDY OF CAPECITABINE IN OXIDATIVE CONDITION (1N H_2O_2):

Procedure

Prepare working standard of 40ppm solution from stored 1000ppm 1N H_2O_2solution with drug dissolved in it by taking 1ml of it into 10ml volumetric flask and adding diluents to it upto mark which gives 100ppm solution and again repeating same procedure by taking 1ml of 100ppm solution to finally prepare 10ppm solution [13].

Inject blank 1N H_2O_2into HPLC and run it.

Then inject 10ppm solution prepared in 1st step into HPLC and run it.

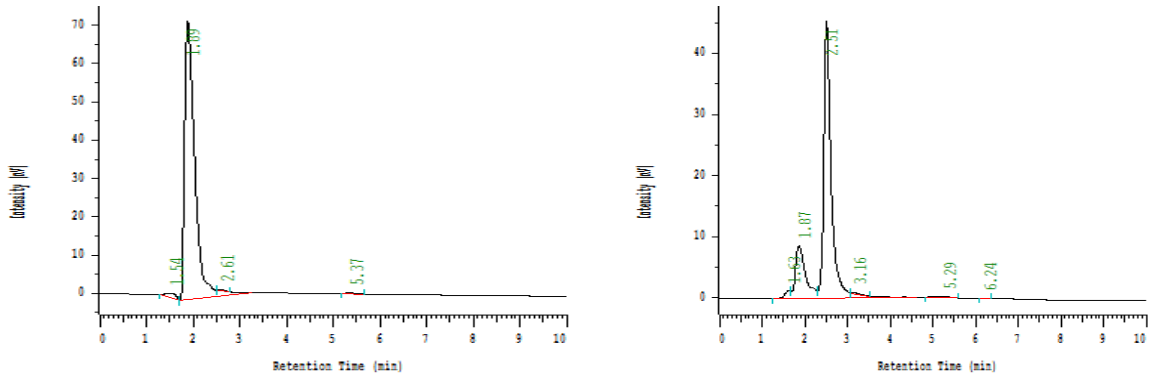


Fig 2.12.1: Chromatogram of 1N Blank H_2O_2 : Chromatogram of stability study of Drug in 1N H_2O_2

Table 2.12.1: Results of Oxidation with 1N H_2O_2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.51	748053	2.04	1924

STABILITY OF CAPECITABINE IN THERMAL CONDITION

Prepare a working standard of 40 ppm from the stock solution and keep in oven at 60℃ for atleast 6 hours to determine its stability. After 6 hours inject the solution and run it [13].

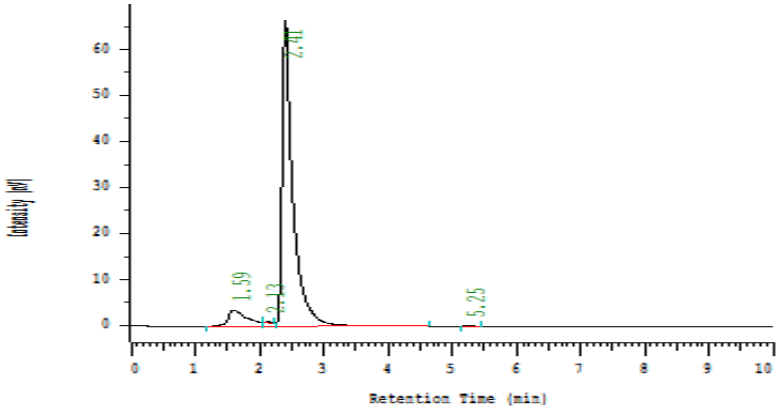


Fig 2.13.1: Chromatogram of stability study in thermal condition:

Table 2.13.1: Results of Thermal hydrolysis

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.41	1209358	1.04	2924

Results of degradation studies

The results of the stress studies indicated the **specificity** of the method that has been developed. Capecitabine was degraded only in 3% H2O2 & temperature stress conditions. The result of forced degradation studies are given in the following table.

Table 2.14.1: Results of force degradation studies of Capacetabine API

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	43.75	54.61	98.36
Basic Hydrolysis (0.I M NaOH)	24Hrs.	43.32	55.02	98.32
Thermal Degradation (50 ^{0}C)	24Hrs.	97.39	----------	97.39
3 % Hydrogen peroxide	24Hrs.	59.75	30.28	100.03

METHOD VALIDATION

Accuracy: Recovery study

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 40µg/ml. From that percentage recovery values were calculated. The results were shown in table-21.

Table 2.15.1: Accuracy Areas

Level of Conc	Conc. Injected	AUC	Conc. Found	% Recovery
80	30	832564	30.15	100.5
80	30	831364	30.11	100.3667
80	30	835243	30.25	100.8333
100	40	1103706	40.45	101.125
100	40	1105698	40.53	101.325
100	40	1107852	40.61	101.525
120	50	1332456	49.14	98.28
120	50	1325698	48.88	97.76
120	50	1332584	49.15	98.3
				100.0017
			Avg.	
				1.470193
			SD	
			%	1.470168
			RSD	

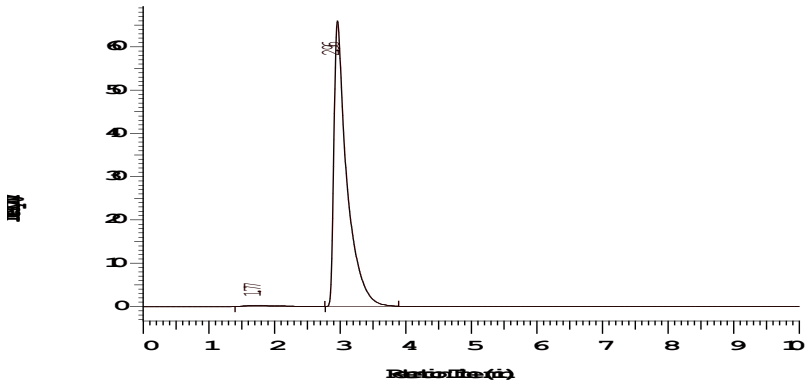


Fig 2.15.1: Chromatogram for accuracy-80%-1

Table 2.15.1: Results of accuracy-80%-1

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	832564	0.84	2245

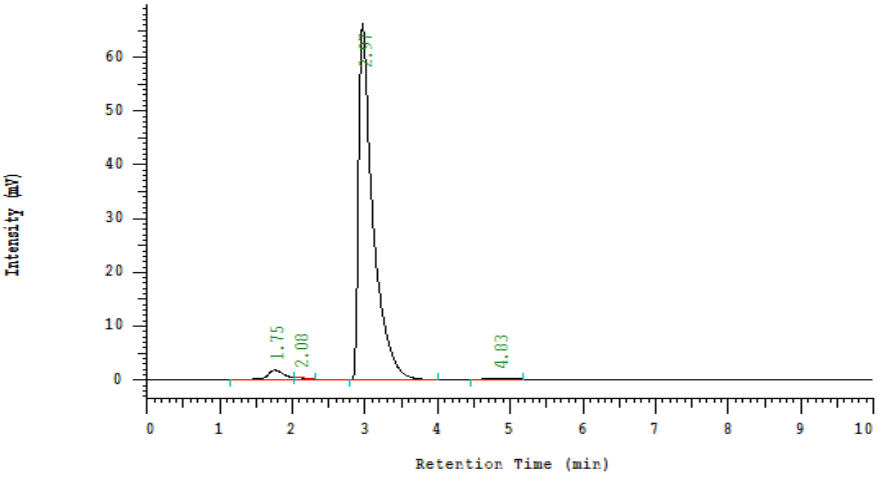


Fig 2.15.2: Chromatogram for accuracy-80%-2

Table 2.15.2: Results of accuracy-80%-2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.97	831364	1.12	2645

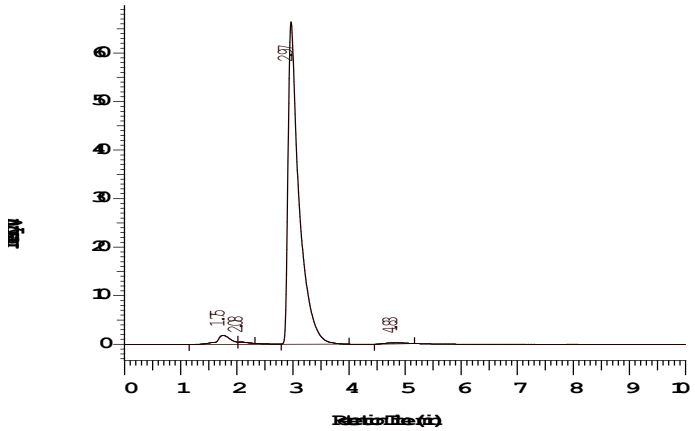


Fig 2.15.3: Chromatogram for accuracy-80%-3

Table 2.15.3: Results of accuracy-80%-3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.97	835243	1.12	2645

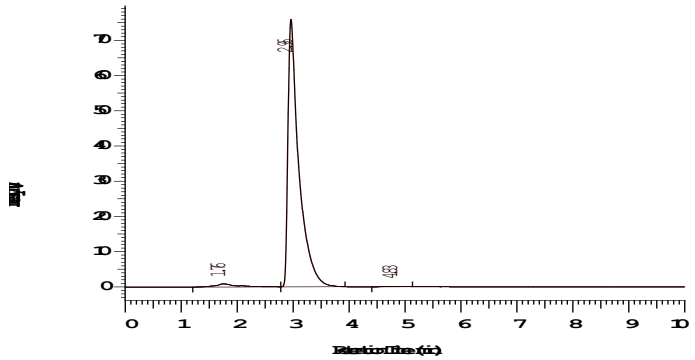


Fig 2.15.4: Chromatogram for accuracy-100%-1

Table 2.15.4: Results of accuracy-100%-1

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	1103706	1.02	2925

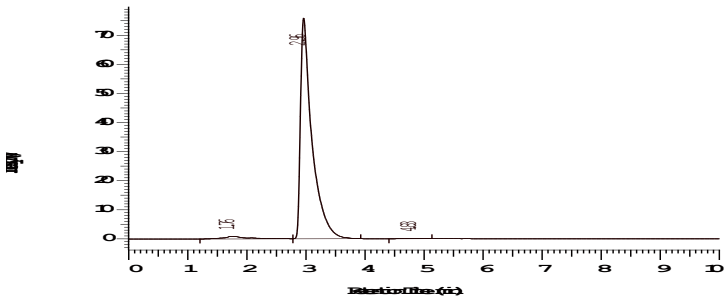


Fig 2.15.5: Chromatogram for accuracy-100%-2

Table 2.15.5: Results of accuracy-100%-2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	1105698	1.02	2925

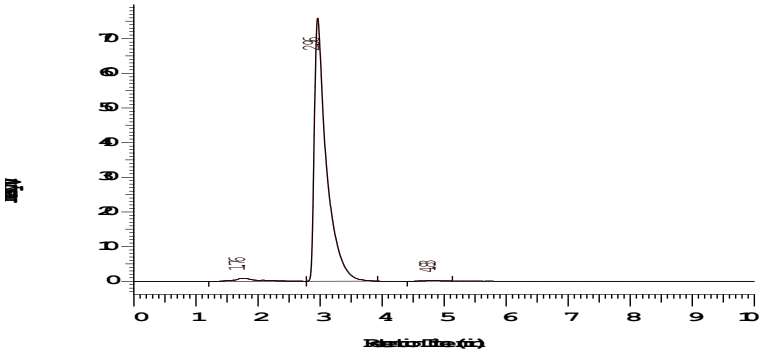


Fig 2.15.6: Chromatogram for accuracy-100%-3

Table 2.15.6: Results of accuracy-100%-3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	1107852	1.05	2955

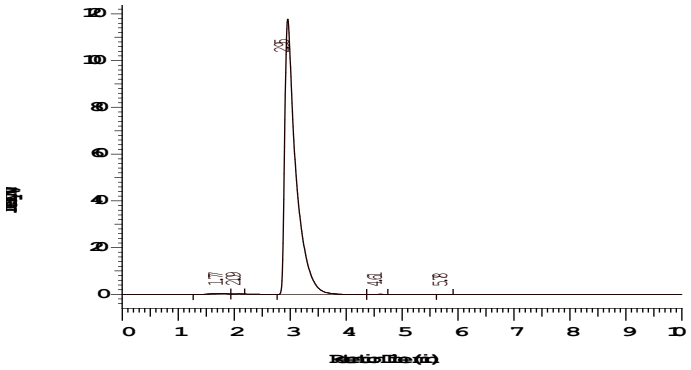


Fig 2.15.7: Chromatogram for accuracy-120%-1

Table 2.15.8: Results of accuracy-120%-1

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.99	1332456	1.05	2845

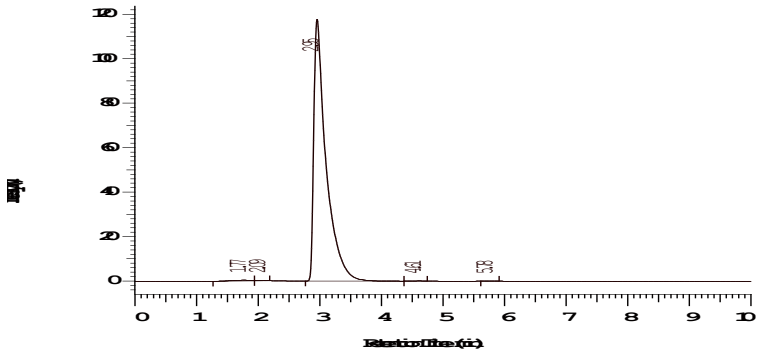


Fig 2.15.9: Chromatogram for accuracy-120%-2

Table 2.15.9: Results of accuracy-120%-2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.95	1325698	1.02	2741

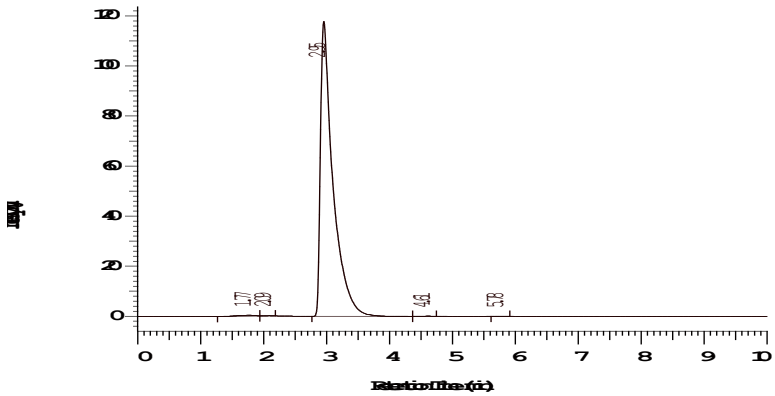


Fig 2.15.10: Chromatogram for accuracy-120%-3

Table 2.15.10: Results of accuracy-120%-3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.95	1332584	1.02	2741

PRECISION

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Capecitabine (API) The percent relative standard deviation were calculated for Capacetabine are presented in the table-31.

Table 2.16.1: Repeatability Results

HPLC Injection Replicates of Capacetabine	Retention Time	Area
Replicate – 1	2.95	1202543
Replicate – 2	2.94	1256603
Replicate – 3	2.97	1246755
Replicate – 4	2.95	1253667
Replicate – 5	2.97	1261099
Average	2.956	1244133
Standard Deviation	0.013416	23826.7
% RSD	0.45387	1.915124

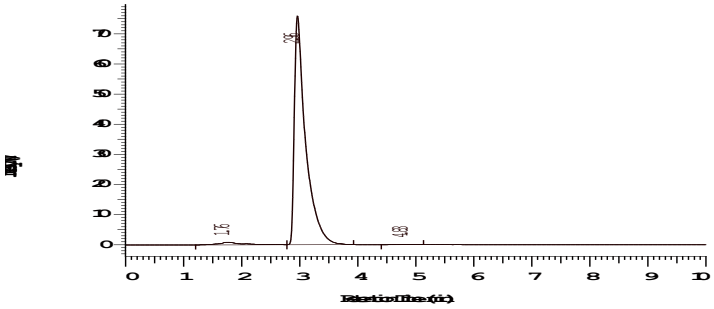


Fig 2.16.1: Chromatogram for Repeatability- 1

Table 2.16.1: Results of Repeatability-1

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.95	1202543	0.98	2441

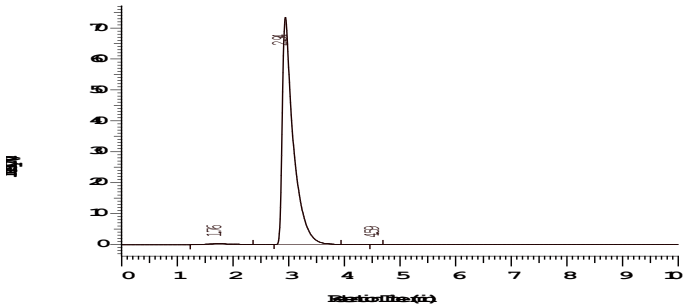


Fig 2.16.2: Chromatogram for Repeatability-2

Table 2.16.2: Results of Repeatability-2

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.94	1256603	0.99	2854

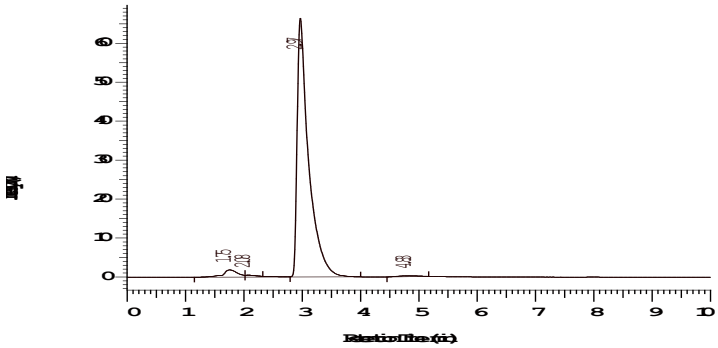


Fig 2.16.3: Chromatogram for Repeatability-3

Table 2.16.3: Results of Repeatability-3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.97	1246755	0.99	2854

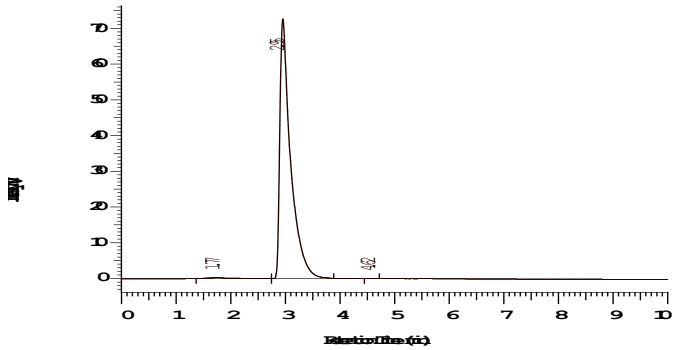


Fig 2.16.4: Chromatogram for Repeatability-4

Table 2.16.4: Results of Repeatability-4

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.95	1253667	0.99	2854

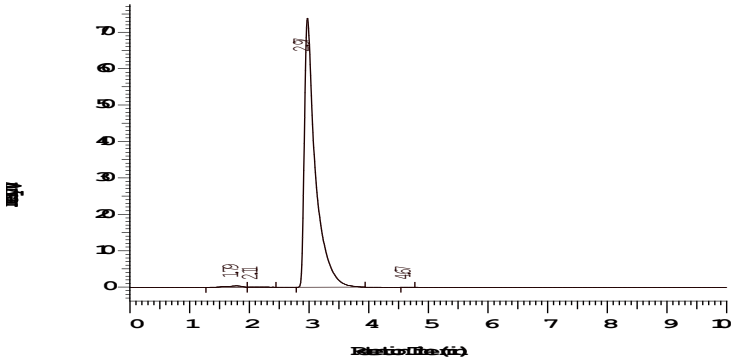


Fig 2.16.5: Chromatogram for Repeatability-5

Table 2.16.5: Results of Repeatability-5

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.95	1261099	0.99	2854

Intra-assay & inter-assay

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 2.17.1: Results of intra-assay & inter-assay

Conc. Of Capacetabine (API) (µg/ml)	Observed Conc. Of Capacetabine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
20	20.01	0.86	20.03	0.87
40	40.02	0.30	40.03	0.32
60	59.97	0.13	59.95	0.11

Linearity & Range

The calibration curve showed good linearity in the range of 0-100 µg/ml, for Capecitabine (API) with correlation coefficient (r^2) of 0.995 (Fig. 33). A typical calibration curve has the regression equation of y = 26326x + 38649 for Capacetabine.

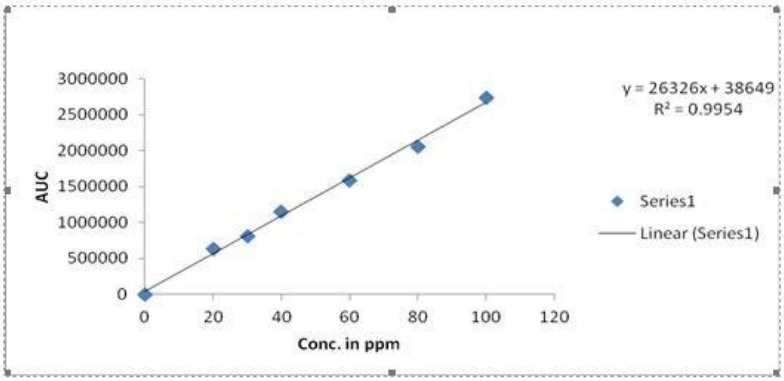


Fig 2.18.1: Calibration curve of Capecitabine (API).

Table 2.18.1: Results of linearity

CONC.	AUC (n=6)
0	0
20	635564
30	804839
40	1144133
60	1581052
80	2055851
100	2736626

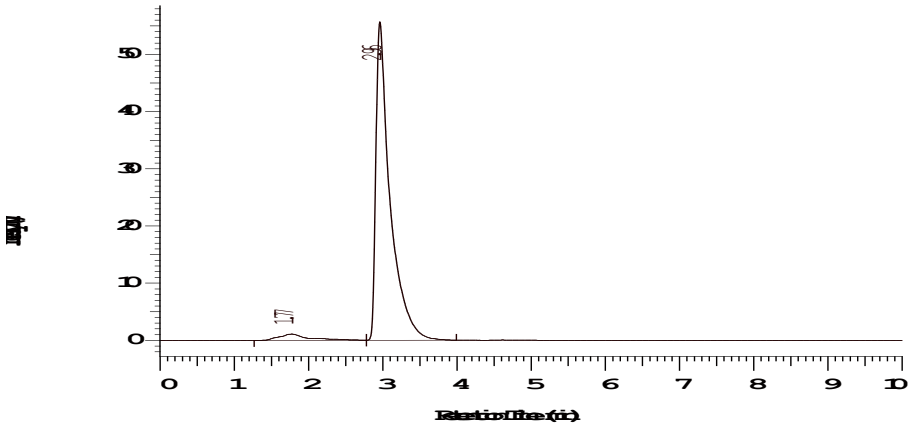


Fig 2.18.2: Chromatogram for 20 ppm

Table 2.18.2: Results of 20 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	635564	1.01	2914

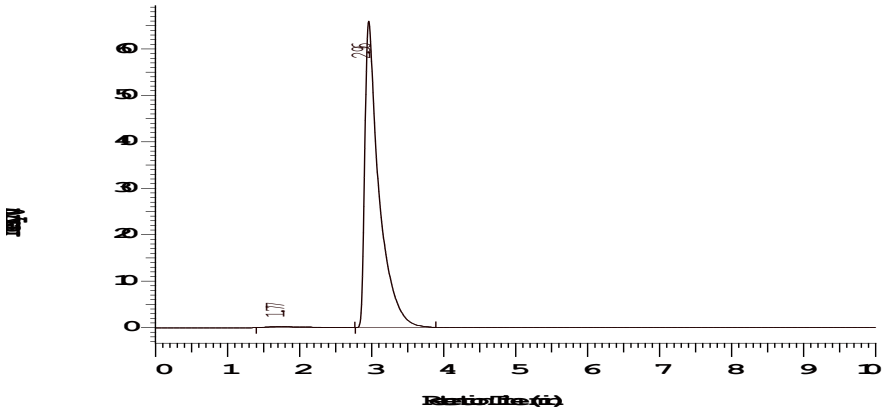


Fig 2.18.3: Chromatogram for 30 ppm

Table 2.18.3: Results of 30 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	804839	1.14	2910

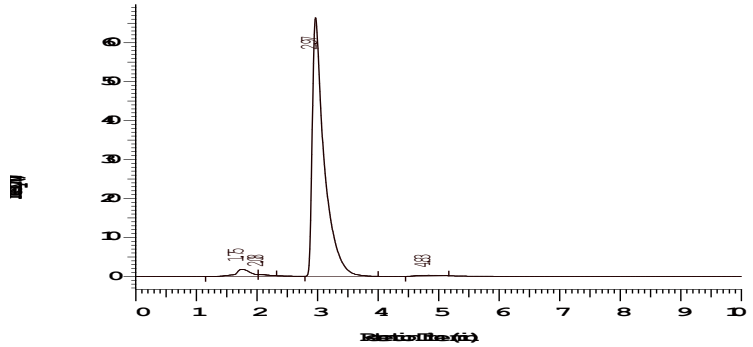


Fig2.18.4: Chromatogram for 40 ppm

Table 2.18.4: Results of 40 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.97	1144133	1.14	2910

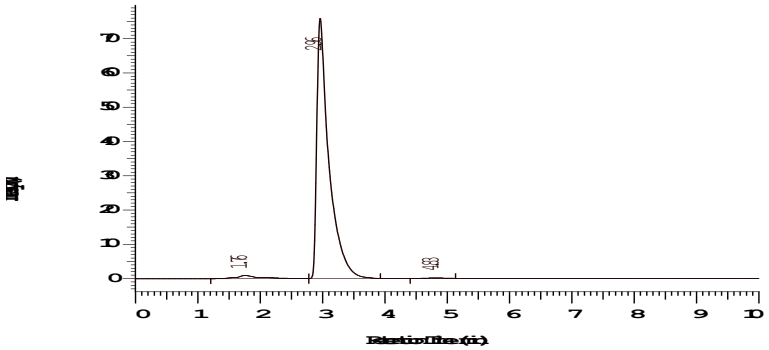


Fig 2.18.5: Chromatogram for 60 ppm

Table 2.18.5: Results of 60 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	1581052	1.15	2921

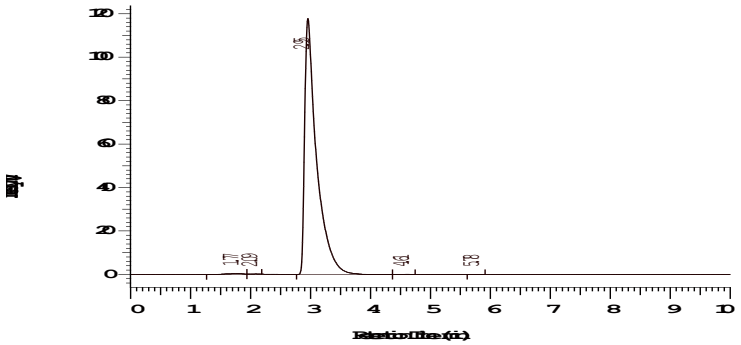


Fig 2.18.6: Chromatogram for 80 ppm

Table 2.18.6: Results of 80 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	2055851	1.15	2715

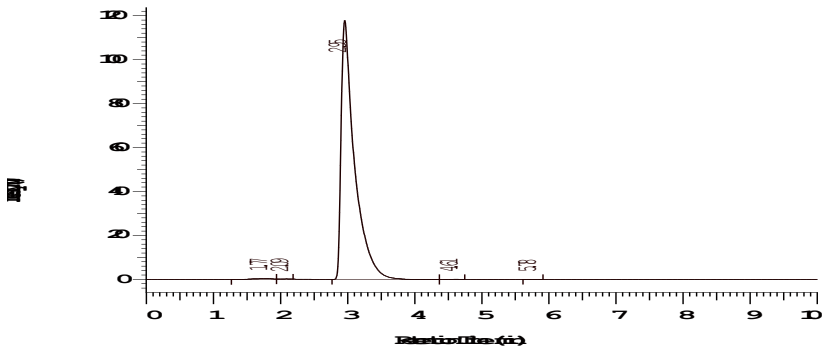


Fig 2.18.7: Chromatogram for 100 ppm

Table 2.18.7: Results of 10s0 ppm

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPECITABINE	2.96	2736626	1.15	2715

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-45, % RSD < 2%) the developed RP-HPLC method for the analysis of Capecitabine(API).

Table 2.19.1: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.06
Flow (0.9 ml/min)	0.04
Temperature (27^{0}C)	0.08
Temperature (23^{0}C)	0.11
Wavelength of Detection (244.5 nm)	0.03
Wavelength of detection (240.5 nm)	0.02

LOD & LOQ

Limit of detection (LOD) and Limit of quantification (LOQ) for Capecitabine were determined by performing various trials at different lowest concentrations. The final LOD and LOQ values at least concentrations were detected by observing signal to noise ratios respectively. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.03 & 0.09 µg/ml respectively.

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Capecitabine, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be highly soluble in acetonitrile and methanol. Drug was soluble in water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Capecitabine it is evident that most of the HPLC work can be accomplished in the wavelength range of 210-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Capecitabine in different formulations a sensitive & selective RP-HPLC method has been developed & validated for the analysis of Capacetabine API.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for purity which can help in the analysis of Capecitabine in API.

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