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METHOD DEVELOPMENT AND VALIDATION OF CAPECITABINE IN TABLETS BY RP-HPLC METHOD

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Abstract:

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Capecitabine in Bulk and its pharmaceutical formulation. Separation was achieved with a Develosil (ODS-MG-5; 100 x 4.6mm I.D; particle size 5 µm) Column and buffer Methanol (450:550) v/v as eluent and purified water, methanol and acetonitrile(600:350:50)v/v as diluent at flow rate 1.0 mL/min and the Column temperature was 40°C. The described method of Capecitabine is linear over a range of 6 µg/mL to 30 µg/mL. The method precision for the determination of assay was below 2.0% RSD.

Key words: *Capecitabine, RP-HPLC, Develosil ODS-MG-5.*

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INTRODUCTION:

Analytical chemistry is defined as "The science and the art of determining the composition of materials, which deals with both theoretical, practical science. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species. Pharmaceutical analysis deals medicaments and their precursors.

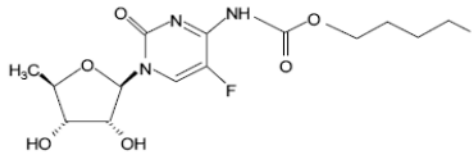
Quality is important in every product. Quality control is a concept, which strives to produce a perfect product. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Physico-chemical methods are optical, photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods.

Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

Chromatography: The term *chromatography* was first used by the Russian chemist and botanist Michael Tswett in 1906. The term *chromatography* is derived from the Greek words: *Chroma* for colour and *Graphein* to write. "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

Drug Profile:



Drug : Capecitabine

Molecular structure :

Molecular formula: C_{15} H_{22} F N_3 O_6

Chemical name: 5'-deoxy-5-fluor[(pentyloxy)carbonyl]-cytidine

Category: Anti-cancer, Antimetabolites, Antineoplastic

Adverse effects: Loss of appetite, Hair loss, Dehydration.

MATERIALS AND METHODS:

Chemicals : Methanol, Acetonitrile, purified water, glacial acetic acid.

Instruments: HPLC (empower-2software), HPLCdetector, PHmeter, Centrifuge, Ultrasonicater, UV Spectrophotometer, Micro Balance, Water Purifier

Methodology:

The parameters used for the developed method and chromatograms were obtained for the drug sample were shown in Table-I.

Table 1: Chromatographic conditions

1	Mobile phase	Buffer (glacial acetic acid) and Methanol in the ratio (450:550)
2	Column	Develosil ODS-MG-5(100x4.6mm), 5µ
3	Flow rate	1.0 ml/min
4	Detector wavelength	250 nm
5	Column temperature	40˚C
6	Injection volume	10 µl
7	Run time	10 min
8	Retention time	5.334 min

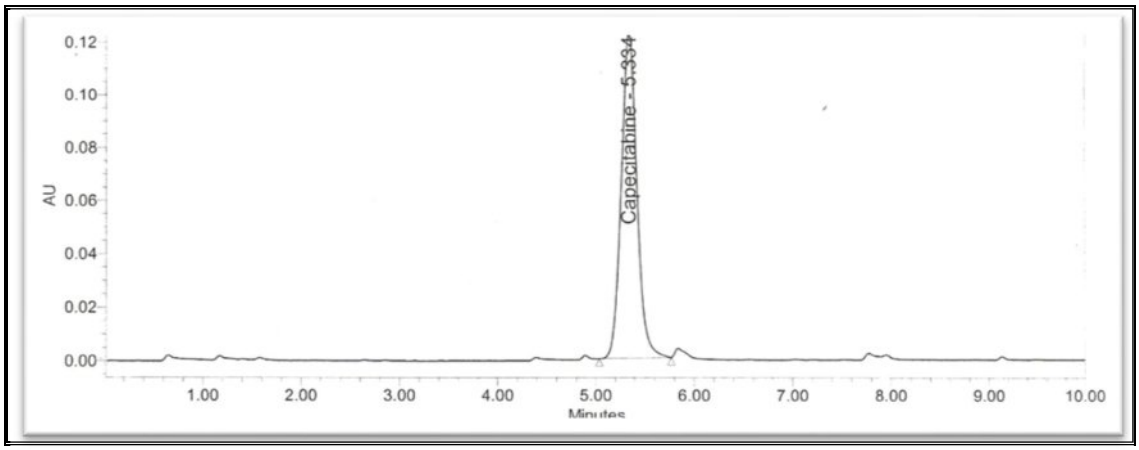


Fig 1: Chromatogram for the trial

Analytical Method Validation:

Preparation of Capecitabine Standard solultion:

Accurately Weigh 60mg of Capecitabine working Standard and add about 600 ml of diluents. Cool the solution to room temperature and dilute to volume with diluents.

Preparation of Sample solution:

Accurately weigh the sample equivalent to 15 mg of Capecitabine into a 250 ml Amber colour volumetric flask. Add about 180 ml of diluents, shake for 10 minutes and sonicate for 20 minutes. Cool the solution to room temperature and dilute to volume with diluents.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank. It should not show any interference from the diluent, excipients at the retention time of analytical peak in of assay method validation**.**

Linearity and Range:

The linearity of the analytical method for assay by injecting the various concentrations of Standard preparation prepared in the range of 6µg/ml to 30µg/ml of test concentration, into the chromatograph, covering 5 different concentrations. Reported the result by intercept and regression coefficient from the plot obtained for Concentration Vs. Peak response of Capecitabine in standard preparation. The range of the analytical method in concentration (%) was be reported (Correlation coefficient should be greater than or equal to 0.999).

Accuracy:

The standard solutions of accuracy 80% - 120% was injected into chromatographic system. Calculate the amount found and amount added for capecitabine and calculate the individual % recovery and mean % recovery values. The % Recovery for each level should be between 98.0 to 102.0%.

Precision:

The precision of the method was determined by repeatability and intermediate precision of the Capecitabine standard solutions. The standard solution was injected for six times and measured the area for all six injections. The % RSD for the area of six standard injections results should not be more than 2.

Limit of Detection and Quantification:

Detection Limit: The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualtitated as an exact value.

Quantitation Limit: The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Robustness:

The Robustness of analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations. The % RSD for the area of six sample injections results should not be more than 2%

Examples of typical variations in assay method validation by HPLC are:

- ✓ Mobile phase flow rate
- ✓ Column temperature
- ✓ Change in wavelength etc

$$\% \text{ Assay} = \frac{TA}{SA} \times \frac{S\,W}{100} \times \frac{250}{TW} \times \frac{P}{100} \times \frac{\text{Avg Wt.}}{LA} \times 100$$

TA = Peak area response due to Capecitabine from sample

SA = Peak area response due to Capecitabine from standard

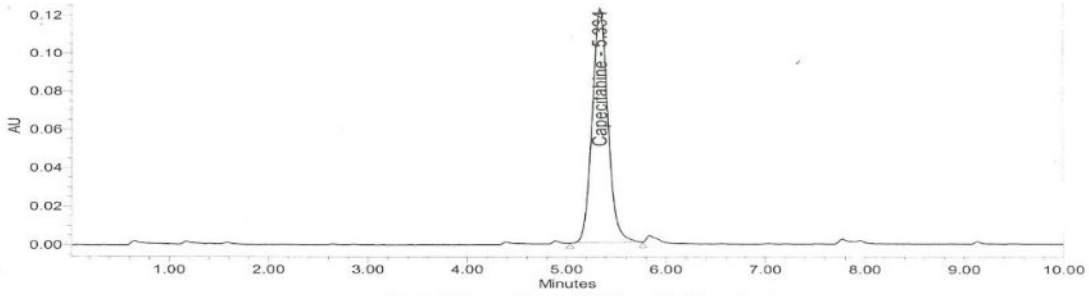
SW = Weight of Capecitabine working standard taken in mg

P = Purity of Capecitabine working standard taken on as is basis

L.A = Labeled amount of Capecitabinein mg

Results:

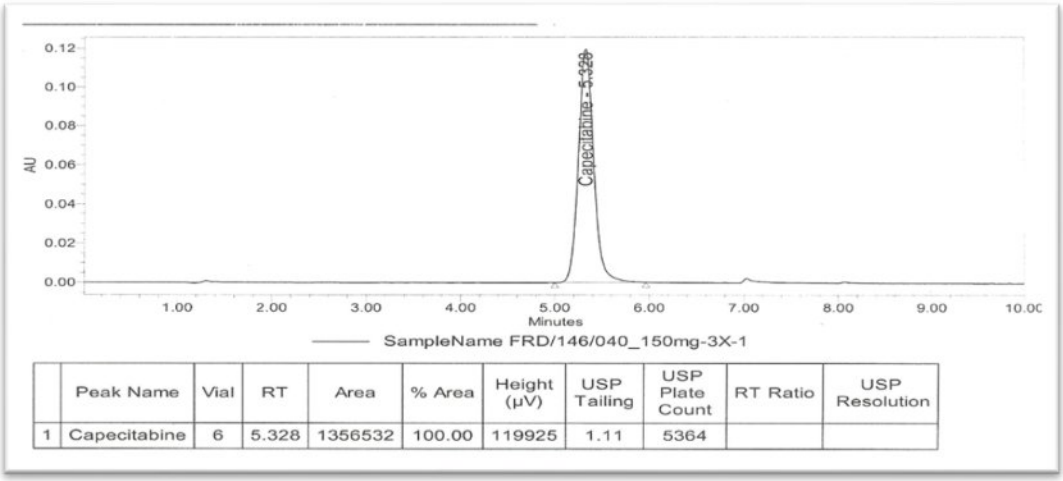
Capecitabine standard:



	Peak Name	Vial	RT	Area	% Area	Height (µV)	USP Tailing	USP Plate Count	RT Ratio	USP Resolution
1	Capecitabine	5	5.334	1364432	100.00	122651	1.10	5431		

Fig 2: Chromatogram showing standard preparation

Capecitabine sample:



	Peak Name	Vial	RT	Area	% Area	Height (µV)	USP Tailing	USP Plate Count	RT Ratio	USP Resolution
1	Capecitabine	6	5.328	1356532	100.00	119925	1.11	5364		

Fig 3: Chromatogram showing sample preparations

Validation:

System Suitability:

The system suitability studies were done with the 60mg of standard drug. The system suitability studies were done with the 60mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.

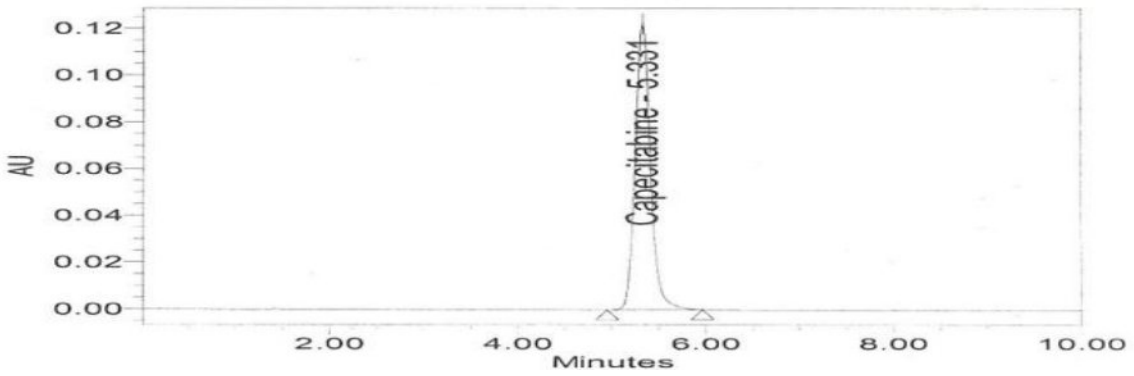


Fig 4: Chromatogram showing system suitability

Table 2: Showing results from system suitability study

S.No	Peak Name	Rt (min)	Area	USP Tailing	Plate count
1	Capecitabine	5.332	1383340	1.12	5413
2	Capecitabine	5.331	1387644	1.12	5377
3	Capecitabine	5.330	1387750	1.11	5396
4	Capecitabine	5.330	1388970	1.11	5385
5	Capecitabine	5.330	1389243	1.11	5369
6	Capecitabine	5.328	1385820	1.12	5364
Mean			1387128	1.12	5384
SD			2217.27		
%RSD			0.16		

Table 3: Summary of system suitability study

System suitability parameters	Results (avg.)
%RSD	0.16
Tailing factor	1.12
Plate count	5384
No. of theoretical plates	4890
Relative retention	…….
Resolution	…….
Capacity factor	…….

Linearity:
The linearity study was performed for the concentration of 6µg/ml to 30µg/ml level. Each level was injected into chromatographic system. The chromatograms are shown in Fig. No.5-9 and results are tabulated in Table. No4. Calibration Curves for capecitabine are shown in Fig.No.10 and results are tabulated.

Linearity level-1 (6µg/ml):

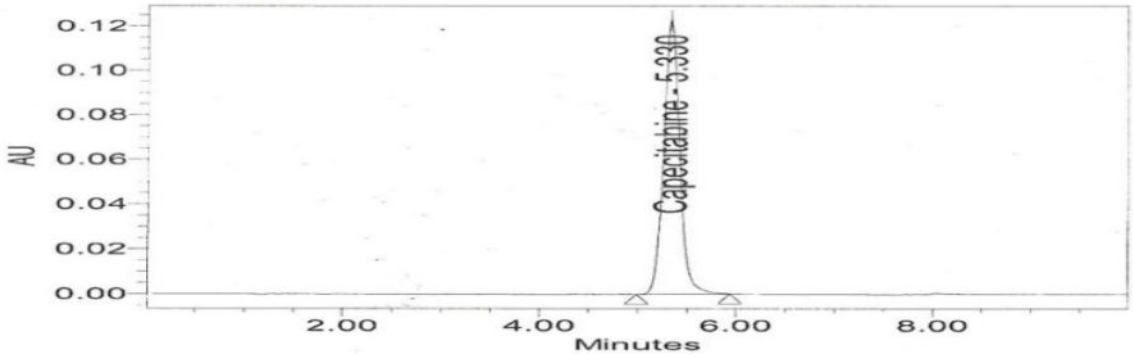


Fig 5: Chromatogram showing linearity level-1

Linearity level-2 (12µg/ml):

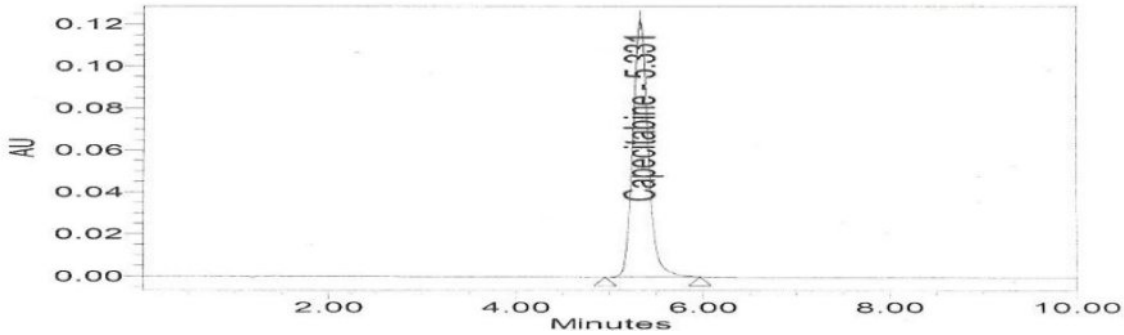


Fig 6: Chromatogram showing linearity level-2

Linearity level-3 (18µg/ml):

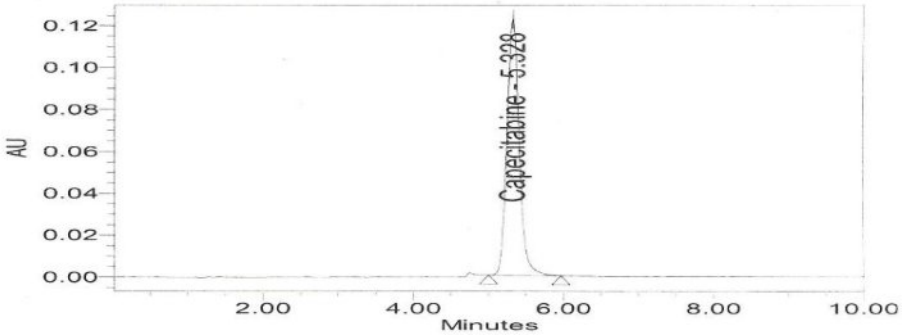


Fig 7: Chromatogram showing linearity level-3

Linearity level-4 (24µg/ml):

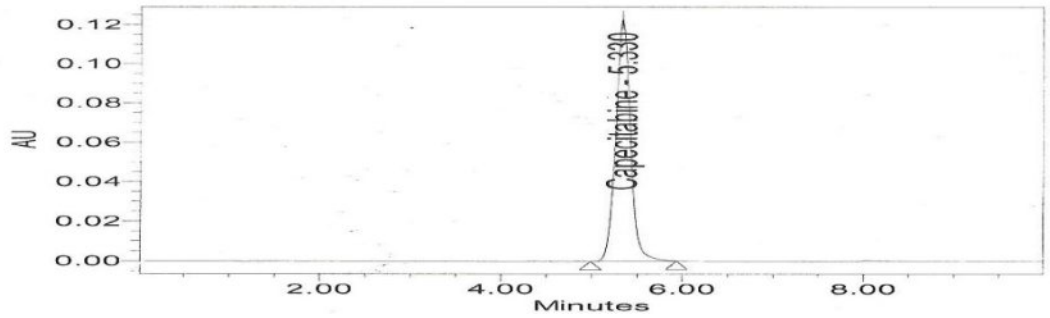


Fig 8: Chromatogram showing linearity level-4

Linearity level-5 (30µg/ml)

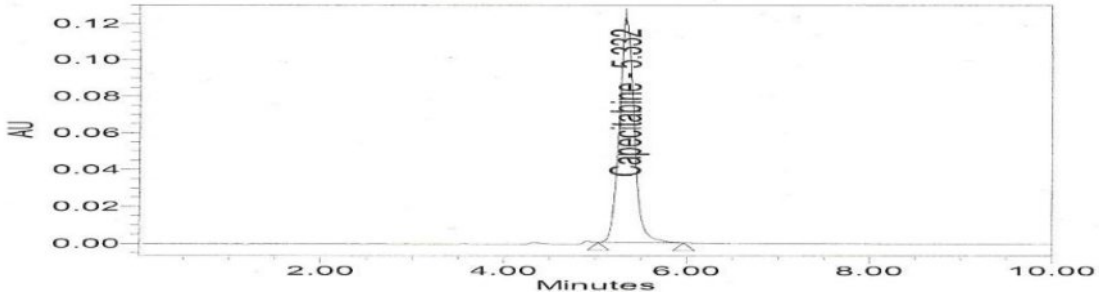


Fig 9: Chromatogram showing linearity level-5

Table 5: showing results from linearity study

S.No.	Linearity Level	Concentration (µg/ml)	Peak area
1	I	6	143119
2	II	12	282164
3	III	18	432216
4	IV	24	572315
5	V	30	692418
Correlation Coefficient			**0.999**

Plotting of calibration curve for Capecitabine:

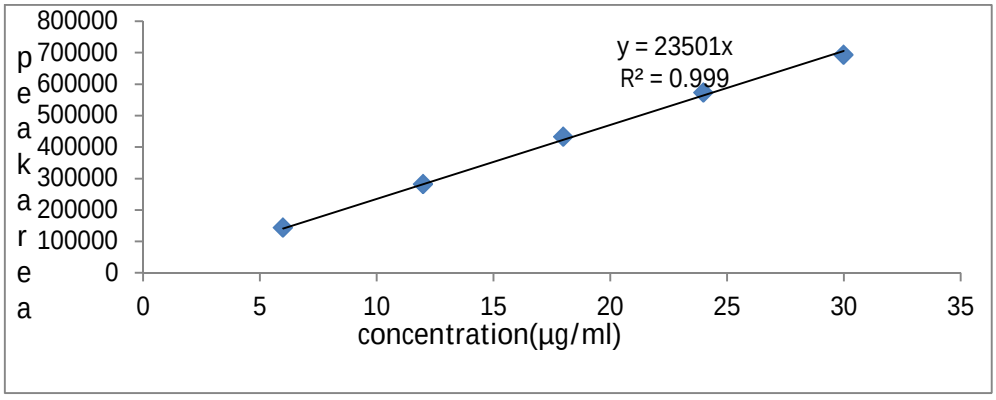


Fig 10: Calibration curve of Capecitabine

The linearity study was performed the correlation coefficient of capecitabine was found to be 0.999 respectively (NMT 0.999).

Specificity:

The system suitability for specificity was carried out to determine whether there is any interferences of any impurities in retention time of analytical peak. The chromatograms are shown in Fig. No.11-12.

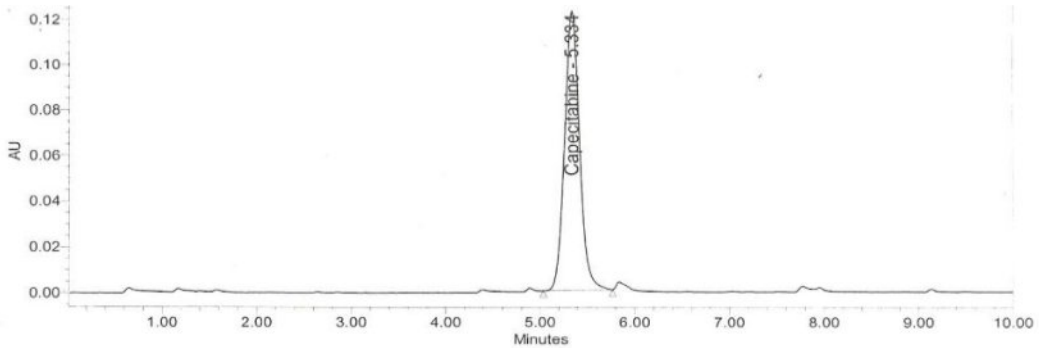


Fig 11: Chromatogram showing standard preparation

Table 6: Showing results from specificity studies of Standard

Sl.No.	Drug name	vail	RT	Peak area	USP plate count	USP tailing
1.	Capecitabine	5	5.334	1364432	5431	1.10

Capecitabine sample:

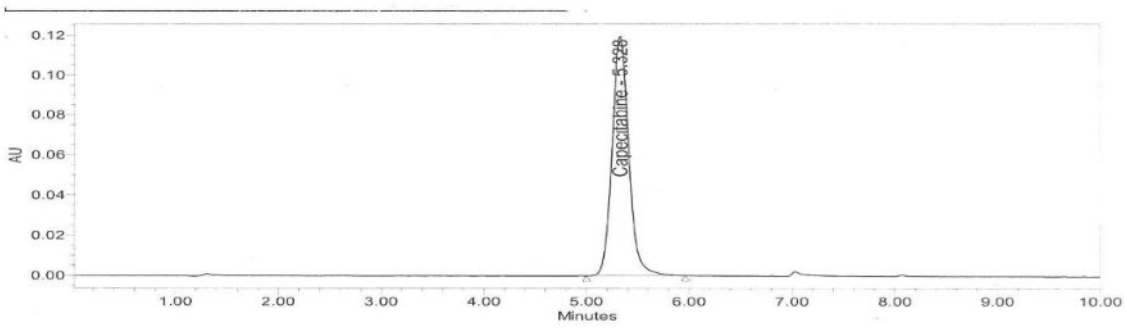


Fig 12: Chromatogram showing sample preparation

Table 7: Showing results from specificity studies of sample

	Drug name	vail	RT	Peak area	USP plate count	USP tailing
1.	Capecitabine	6	5.328	1356532	5431	1.10

It was found that there was no interference of impurities in retention time of analytical peak. The method show excellent specificity with capecitabine eluting at retention of 5.328 minutes.

Accuracy:

The accuracy study was performed for 50%, 100% and 150% for capecitabine. Each level was injected in triplicate into chromatographic system. Chromatograms are shown in Fig.No.13-15 and results are tabulated in Table. No.8

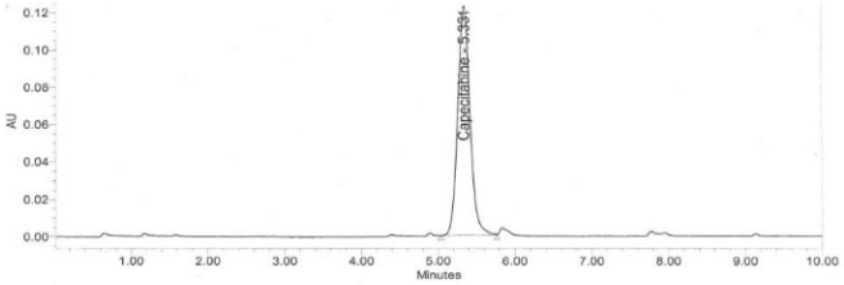


Fig 13: Chromatogram showing accuracy-100% injection

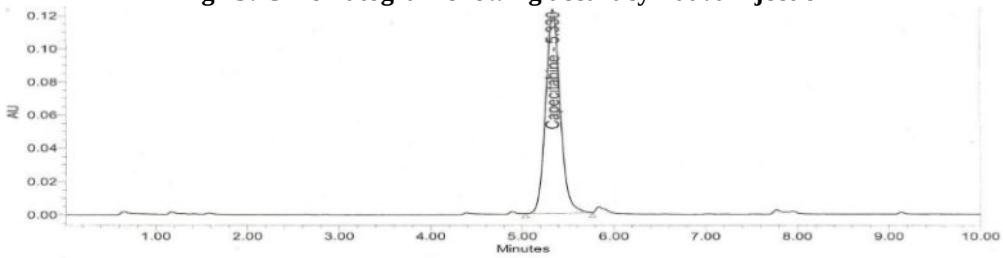


Fig 14: Chromatogram showing accuracy-80% injection

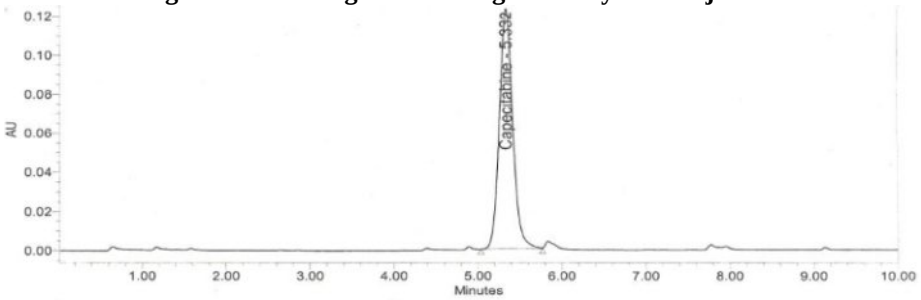


Fig 15 : Chromatogram showing accuracy-120% injection

Table 8: Showing result from accuracy study

Level of % recovery	Amount of drug spiked(µg/ml)	Drug recovered	%Recovery	Mean	SD	%RSD
80	9.6	9.62	100.2	100.4	0.346	0.34
		9.62	100.2			
		9.68	100.8			
100	12	12.23	101.9	101.6	0.974	0.95
		12.08	100.6			
		12.31	102.5			
120	14.4	14.26	99.02	99.70	0.6451	0.64
		14.21	99.8			
		14.45	100.3			

The accuracy study was performed for % recovery. The % recovery was found to be 100.4 to 99.70% respectively. (NLT 98% and NMT 102%).

Precision:

Repeatability:

The precision study was performed for six injections of capecitabine. Each standard injection was injected into chromatographic system and area was used for calculation of %RSD. The chromatograms are shown in Fig.Nos.16-17 and results are tabulated in Table.Nos.9&10.

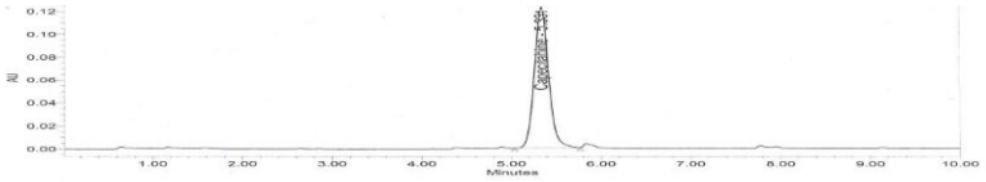


Fig 16: Chromatogram showing system precision

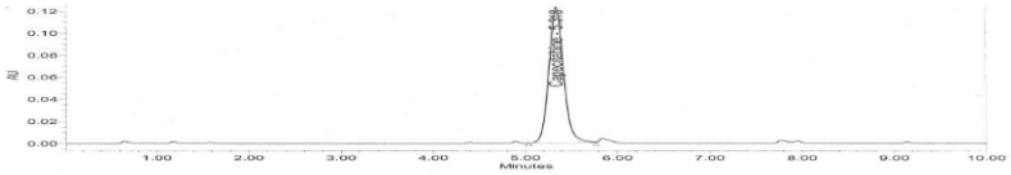


Fig 17: Chromatogram showing method precision

Table 9: Precision study-repeatability (60 µg/ml): Method precision

S.No	Peak Name	Peak area
1	Capecitabine	1381620
2	Capecitabine	1384273
3	Capecitabine	1382656
4	Capecitabine	1383288
5	Capecitabine	1388610
6	Capecitabine	1382144
Mean		1383765
SD		1502.76
%RSD		0.10

Table 10: Precision study-repeatability(60µg/ml): System precision

S.No	Peak Name	Peak area
1	Capecitabine	1382136
2	Capecitabine	1385243
3	Capecitabine	1386230
4	Capecitabine	1386790
5	Capecitabine	1384273
6	Capecitabine	1385280
Mean		1384992
SD		1648.33
%RSD		0.12

Ruggedness:

Intra-day precision:

Intra-day precision was carried out on same day, same HPLC system, using same column at different times.

Inter-day precision:

Inter-day precision was carried out on same HPLC system, using same column on another day. %R.S.D. for 6 replicate injections of standard drug solutions not more than 2.0.Relative standard deviation of % Assay results should not more than 2.0%.

Table 11: Showing from precision study- Intraday

Conc µg/ml	Peak area	Statistical parameters
40	912546	Mean:915887
	916382	S.D:3123.5
	918734	%R.S.D:0.34
60	1364876	Mean:1366257
	1366208	S.D:1407.15
	137689	%R.S.D:0.10
80	1814786	Mean:1816049
	1816124	S.D:1227.72
	1817238	%R.S.D:0.06

Table 12: Showing from precision study- Interday

Conc µg/ml	Peak area			Statistical parameters
	Day-1	Day-2	Day-3	Mean:915780
40	912436	916257	918648	S.D:3133.3
				%R.S.D:0.34
60	1364926	1365182	1367394	Mean:1365834
				S.D:1357.0
				%R.S.D:0.09
80	1814954	1816242	1817438	Mean:1816211
				S.D:1242.28
				%R.S.D:0.07

The precision of method was determined by replicate injection of sample solution. The %RSD of area of intraday precision are 0.3%, 0.10% and 0.06%. %RSD of interday precision was found to be 0.3,0.09% and 0.07%. Precision results are within the limits. (NMT 2).

Limit of Detection and Quantification:

Detection Limit:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank = 42.43 µV

Signal Obtained from LOD solution = 0.00948 µV

LOD = 3.3×σ/s = 3.3×0.00948/42.43 = 0.000737

S/N Ratio value shall be 3 for LOD solution.

Quantitation Limit:

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank = 42.43 µV

Signal Obtained from LOQ solution = 0.00948 µV

LOD = 10×σ/s = 10×0.00948/42.43 = 0.02342

S/N Ratio value shall be 10 for LOQ solution.

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was done by changing the flow rate (± 1), column temperature (± 5°C), Changing the wavelength (± 5 nm). The %RSD of peak area, tailing factor and theoretical plates of Capecitabinestandard was found within the limits.

Influence on variation of Flow rate:

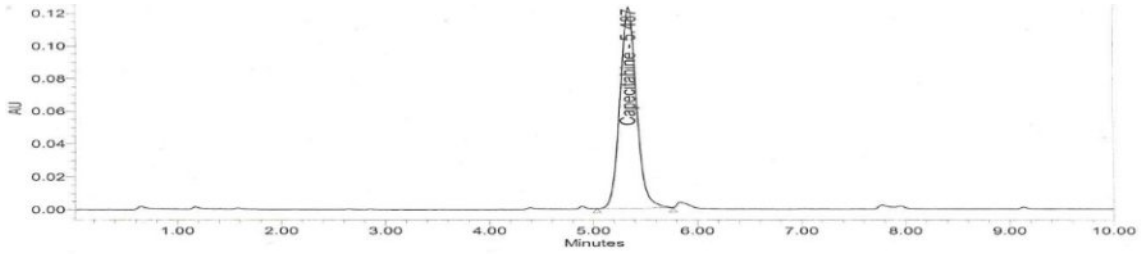


Fig 18: Chromatogram showing variation of flow rate

Table 13: Showing results from robustness study

Replicate standard injections at 0.9ml\min			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364216	Average :1364003 % RSD = 0.11	% RSD : not more than 2%
2	1362325		
3	1365470		

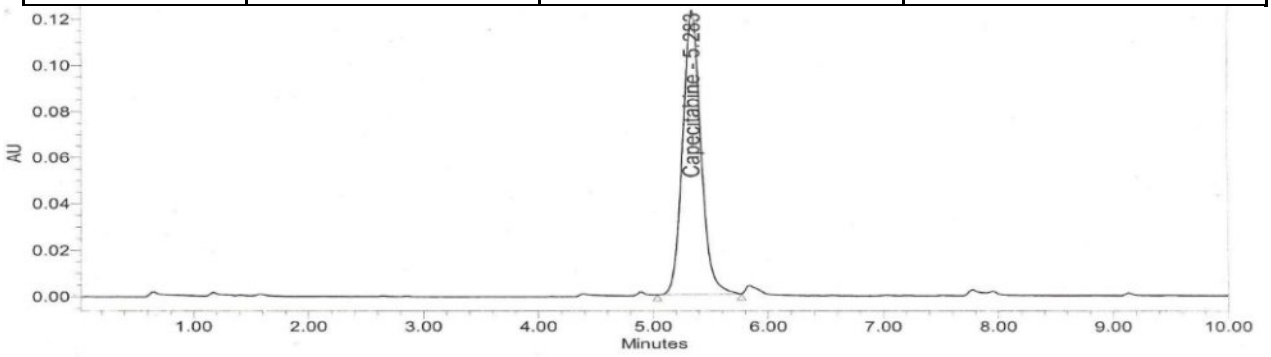


Fig 19: Chromatogram showing variation of flow rate

Table 14: Showing results from robustness study

Replicate standard injections at 1.1ml\min			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1384273	Average :1385179 % RSD = 0.14	% RSD : not more than 2%
2	1388610		
3	1382656		

The analytical method was found to be robust with respect to change in flow rate.

Influence on variation of Column Temperature:

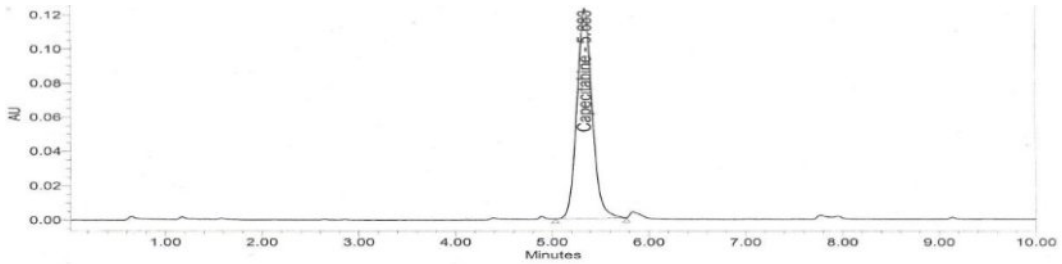


Fig 20: Chromatogram showing variation of temperature

Table 15: Showing results from robustness study

Replicate standard injections at 35^{0}c			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364354	Average :1366608 % RSD = 0.10%	% RSD : not more than 1%
2	1367124		
3	1368346		

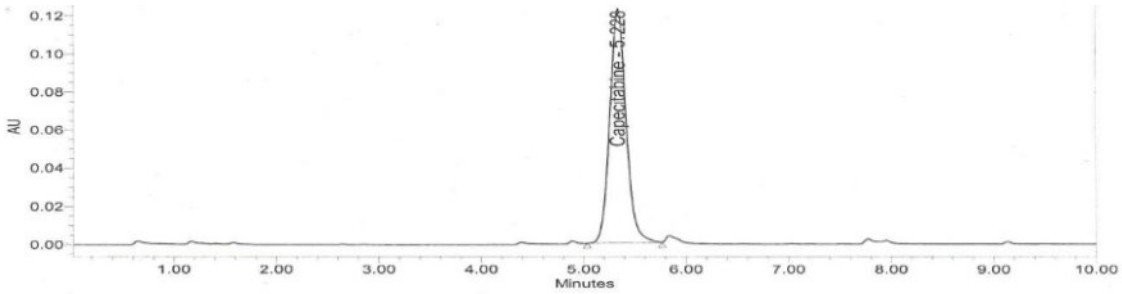


Fig 21: Chromatogram showing variation of temperature

Table 16: Showing results from robustness study

Replicate standard injections at 45^{0}c			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364592	Average :1366723 % RSD = 0.15%	% RSD : not more than 1%
2	1366831		
3	1368746		

Influence on variation of wave length:

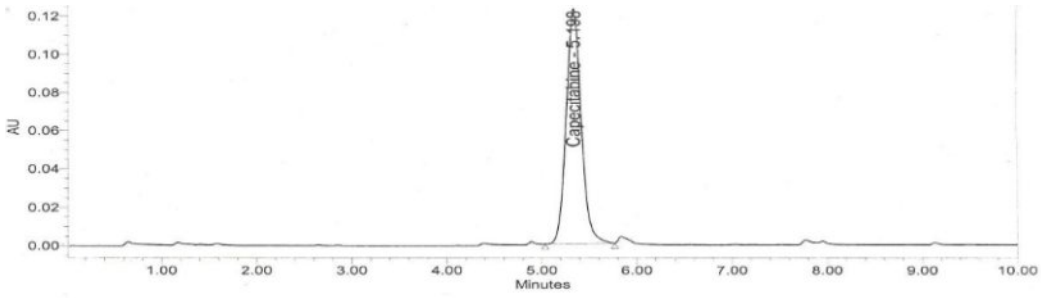


Fig 22: Chromatogram showing variation of wave length

Table 17: Showing results from robustness study

Replicate standard injections at wave length 245 nm			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364234	Average :1364798 % RSD = 0.04	% RSD : not more than 1%
2	1365173		
3	1364986		

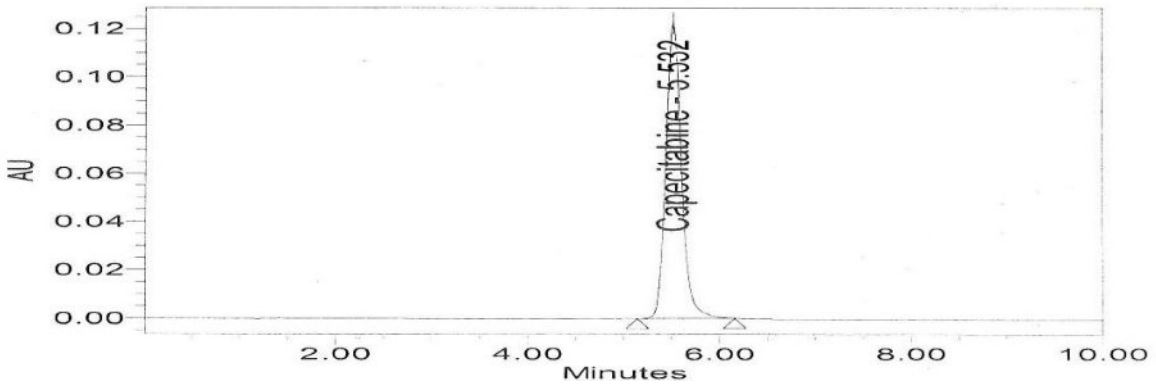


Fig 23: Chromatogram showing variation of wave length

Table 18: Showing results from robustness study

Replicate standard injections at wave length 255nm			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1365216	Average :1366677 % RSD = 0.09	% RSD : not more than 1%
2	1366528		
3	1368287		

Assay calculation:-

$$\%Assay = \frac{??}{??} \times \frac{??}{???} \times \frac{???}{??} \times \frac{?}{???} \times \frac{???.??}{??} \times 100$$

Where,

TA = Peak area response due to Capecitabine from sample

SA = Peak area response due to Capecitabine from standard

SW = Weight of Capecitabine working standard taken in mg

P = Purity of Capecitabine working standard taken on as is basis

%Assay = 1356532/1364432x 60/100x250/192x99.9/100x192/150x100 **= 99.32%**

SUMMARY AND CONCLUSION:

Summary:

Validation Parameter	Acceptance Criteria	Results
System Suitability	The RSD Should be **NMT2%** for each peak	Capecitabine 0.16%
Specificity	The interference of the diluents/placebo is considered insignificant, if the chromatogram of the placebo shows no peak, at the retention time of analyte peak	No peaks are eluted at the retention time of Capecitabine.
Precision		
Method repeatability	The %RSD of 5 replicate injections should be **NMT 2.0%**	%RSD of 6 samples of capecitabine tablets is 0.12for system precision and 0.10 for method precision
Intermediate precision	The% RSD calculated on 6 determinations of assay value should be NMT 2%	The above results are within limits.
Linearity	The correlation coefficient should be NLT **0.9998**	0.9999
Accuracy	The method is considered accurate,if average recovery is NLT 98% andNMT 102%.	Accuracy for the average of triplicate in each concentration samples are within the limit.
Robustness	The system suitability parameters should not vary with method parameters during robustness study.	Test results are within the limits.

CONCLUSION:

A new method has been established for estimation of Capecitabine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Capecitabine by usingDevelosil ODS-MG-5 column, flow rate was 1.0ml/min, mobile Phase: Buffer and Methonal (450:550v/v) and Diluent mixture of purified water, Methanol and Acetonitrile (600:350:50). Detection wave length was 250nm.The instrument used was WATERS HPLC auto sampler. The retention times were found to be 5.334 mins. The correlation coefficient (r^2) was found to be 0.999, % recovery was 100.4-99.70% and %RSD for precision on replicate injection was 0.10 and intermediate precision for intraday precision at condition-I,II and III was 0.3, 0.10 and 0.06 % interday precision at condition-I,II and III was 0.3,0.09 and 0.07% respectively. The precision study was precise, robust, and repeatable. LOD value was 0.000737 and LOQ value was 0.02342.

Hence the method can be used for routine analysis of Capecitabine in API and tablet dosage form

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