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VALIDATED STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF CAPECITABINE

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ABSTRACT: A new stability-indicating high-performance liquid chromatographic method of analysis of capecitabine in pharmaceutical dosage form was developed and validated. The solvent system consisted of methanol: water (60:40% v/v). The retention time of capecitabine was 7.927 min at a flow rate of 1 ml/min on C-18 (Quails BDS, 250 × 4.6 mm, 5µ). Capecitabine was detected at 240 nm. The linear regression analysis data for the linearity plot showed good linear relationship with the correlation coefficient value, R^2 = 0.9991 in the concentration range 4-50 µg/ml. The method was validated according to the ICH guidelines. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with Recovery 98.12-101.61%. The assay of capecitabine was determined in tablet dosage form. The method was also found to be robust. The drug was subjected to stress conditions of acidic, basic, oxidation, photolytic and thermal degradation, and considerable degradation was found in all stress conditions. The method has proven specificity for the stability-indicating method.

INTRODUCTION: Capecitabine is an antineoplastic carbamate of fluoropyrimidine, and it is used in the category of anti-metabolites medicinal drugs. Capecitabine is a chemotherapy agent orally used to treat metastatic and colorectal cancers. Capecitabine is a 5'-deoxy-5-fluorouridine (5'-DFUR) drug which inhibits the synthesis of DNA and slowns the growth of tumor-tissue in cells. This induces the pharmacodynamic response by representing a natural nutrient in cells necessary for the growth of cancer cells. Capecitabine is taken up by the cancer cells and then impairs their growth [1-4]. The literature review indicates that few experimental approaches have been used to measure capecitabine by HPLC [5-8]. **Table 1** shows the list of formulations of capecitabine available in market.

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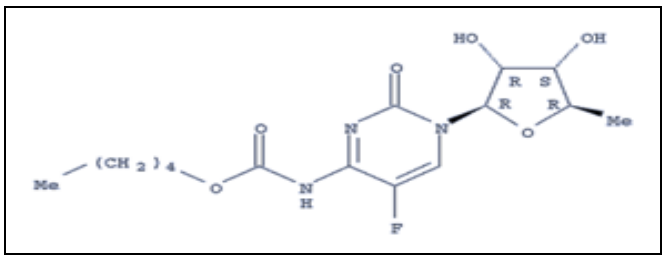
Drug Profile of Capecitabine: [9]



DRUG PROFILE OF CAPECITABINE

Molecular formula	:	$C_{15}H_{22}FN_3O_6$
Structure	:	Capecitabine
Chemical 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
Molecular weight	:	359.3501 g/mol
Description	:	White to off-white crystalline powder
Solubility	:	Soluble in water and slightly soluble in methanol
Melting Range	:	110-121 °C
pka	:	12.59
Category	:	Anti-neoplastic agent, Anti-metabolites and prodrugs
Pharmacokinetic Data		
Bioavailability	:	Extensive
Protein binding albumin)	:	less than 60% (mainly
Metabolism	:	Hepatic, to 5´ - DFCR, 5´ - DFUR (inactive); neoplastic tissue, 5´ -DFUR to active fluorouracil
Half-life	:	45-60 min
Excretion	:	Predominantly excreted in urine
Dosage form	:	Tablet
Dose	:	150 mg, 500mg
Wavelength (λ_{max})	:	239nm
Official Monograph	:	USP

TABLE 1: LIST OF FORMULATIONS OF CAPECITABINE AVAILABLE IN MARKET

S. no.	Brand name	Dose	Dosage form	Manufacturer
1	XELODA	150mg/500mg	Tablet	Roche
2	XABINE	500mg	Tablet	Ranbaxy
3	ZOCTAB	500mg	Tablet	Dabur
4	CAPEGARD	500mg	Tablet	Cipla
5	CAPIIBINE	150mg/500mg	Tablet	Dr .Reddy´s
6	CACIT	500mg	Tablet	Biochem
7	CAPEBIN	500mg	Tablet	Oncare

EXPERIMENTAL:

Chemicals and Reagents: HPLC grade methanol and water were purchased from Merck Company, Mumbai, India.

Method Development:

Preparation of Standard Drug Solutions: Stock solution of Capecitabine was prepared by dissolving 10 mg of Capecitabine in separate 10 mL of the volumetric flask with a small quantity of water. The mixture was sonicated for about 10 min and then made up to volume with water. From the stock solution, 100 µg/mL of capecitabine was prepared.

Chromatographic Conditions: The mobile phase consisted of methanol, and water was filtered before use by means of a 0.45µ membrane in a ratio of 60 to 40 composition of the mobile phase. The mobile phase was supplied with a flow rate of 1.0 ml/min from the solvent reservoir into the tank, and the injection volume was 20 µl. The temperature of the column was kept at 23 ± 10 °C. At 239 nm, eluent controls were performed. The optimized conditions were shown in **Table 2**.

Calibration of Standards: The standard calibration line was constructed for capecitabine. In order to achieve a concentration of 4-50 µg/ml of different capecitabine volumes of the stock, solutions have been correctly transferred to 10 ml volumetric cylindrical flasks. The reference line was achieved by diagraming the peak area of product concentration.

Method Validation: The method was validated according to the ICH guidelines. The following validations characteristics were addressed: specificity and selectivity, linearity, assay, accuracy, precision, limits of detection and quantitation, robustness, ruggedness [10-12].

Specificity and Selectivity: Due to the presence of other excipients, the specificity of the method was assessed for interference. The figure shows that the medicinal product was clearly separated. This study therefore selects the HPLC method presented.

Linearity: The linearity of calibration curves in a pure solution (peak area *vs*.) was tested over the 4-50 µg/ml range. The regression lines for standard drug concentrations using an analysis of regression were lined with the calibration curves and y= 53752x+ 48825, r^2 = 0.991 for capecitabine. Total eluting time was less than 10 min. The mean ± standard difference (SD) was determined for the pitch, intercept, and correlation coefficient of the standard curves (N=3). **Table 3-4** and **Fig. 1** presented the data.

Precision: Six duplicate measurements of 20 µg/mL capecitabine concentration were tested for process accuracy; the results shown in **Table 23**. In order to determine process precision, intraday and inter-day measurements (days 3) of the drug were performed, using 8 µg/mL, 20 µg/mL, 40 mL, intra-day, and inter-day samples were calibrated with a

standard curve simultaneously prepared during the day of testing by calibrating percent RSD for replicate samples (n=3). **Table 7-8** revealed the intra-and inter-day accuracy tests.

Assay: The analysis was carried out by weighing 10 comprises a finely powdered tablet powder equivalent to the 10 mg of API and was precisely weighed. This was obtained with 10ml flask water and added water to label the solution with filtering paper from Whatman 1 (1000 mcg/ml). The volume was added with the moving phase (100mcg/ml), and 1ml was transferred into a 10 ml volumetric flask, then moved to the 10 ml volumetric flask, and the volume upgraded to the mark with a moving phase (10 mcg/ml). The solution was sonicated for 15min, and the total area was measured under above chromatographic conditions.

The analysis process was done triple, and sample weight was determined for the test. **Table 5** measured and displayed the percentage of products present in type, medium, and standard deviation.

Accuracy: Recovery tests have determined the reliability of the system. The drug's reference levels have been applied to the term at the rate of 50%, 100%, 150%. Recovery experiments have been conducted three times, and **Table 6** and **Fig. 2** have measured the percent recovery and relative standard deviation from the capecitabine recovery.

Limits of Detection and Quantitation: Samples containing very low analyte levels are carried out in compliance with the ICH Guidelines for the LOD and LOQ (Limit of Quantitation) detection for the procedure. Using the mathematical formulation process, LOD has been represented by setting the minimum level to reliably detect the analyte. LOQs in the criteria that can be calculated reproducibly with reasonable precision is known as the lowest concentration of analytes. **Table 10** showed the LOD and LOQ values for Capecitabine.

Robustness: The method's robustness was assessed through examination of system suitability requirements and measurement of system suitability parameters by varying HPLC pump flow rates (±0.2%) and organic solvents (±2%) and mobile phase 60. No changes in the R.S.D. peak area, USP tailing factor and conceptual tiles caused any significant changes. While retention periods were much longer and statistical improvements were still possible. **Table 9** revealed the results.

System Suitability: Tests are characterized as the methods that can produce an appropriate precision and accuracy. Following the development and validation of the process, the device suitability was performed. This was done by measuring parameters such as plate (N), resolution (R), tailor, capacity factor, HETP, sample maximum symmetry, and displayed in **Table 11-12**.

Stress Degradation Studies of Capecitabine:

Acid Degradation Studies: Acid decomposition studies were carried out in 0.1 N HCl at room temperature for a period of 3 days. 10mg of the standard capecitabine was weighed and transferred to separate 10 ml standard volumetric flasks. The drug was dissolved by adding water to get the final conc. of 1000µg/ml. From the above solutions, 1ml of capecitabine solution was drawn in to separate 10 ml std flasks and made up to the volume with 9 ml of 0.1N HCl to get the conc. of 100 µg/ml.

From this solution, 2 ml of capecitabine was drawn at time periods of 0 h, 1 h, 2 h, and 4 h, then added 4 ml of mobile phase and pH was adjusted to 4.0 with OPA. The volume was developed and injected with up to 10 ml mobile phase. As a controller, Blank was used (sample solution without the medicine) to determine mobile phase interference. **Table 13-14** showed the findings.

Alkaline Degradation Studies: The analysis of alkaline decomposition at room temperature was carried out in 0.1 NaOH. The Capecitabine formulation weighed 10 mg and was moved to a generic 10ml volumetric container. The drugs are dissolved in water to achieve the final concentration of 1000 µg/ml 1 ml of capecitabine from the above solutions were drawn in regular 10ml containers, and the conc volume was made up of 0.1N NaOH 9 ml 100 µg/ml 100 µg. The 2 ml Cacpecitabine solution has been drawn at 0 h, 1 h, 2 h, and 4 h, and then 4 ml of the mobile stage has been applied, and the PH with OPA has been changed to 4.0. The amount was up to 10 ml and injected into the mobile phase. As a control, blank (sample solution without medicine) has been used, and mobile phase interference has been determined. **Table 15-16** showed the results.

Neutral Condition: A separate 10-ml volumetric flask was drawn from the stock solution for standard capecitabine 1 ml, which is composed of the amount of 9 ml water for finally conc. 100 µg per ml. 1 ml of capecitabine was drawn and injected from these solutions into 10 ml volumetric flask, with a volume of 0 h, 1 h, 2 h, and 4 h.

Oxidative Degradation Studies: In order to achieve a final conc. of 10 ml volumetric flask, 1ml each was drawn from a stock solution with standard Capecitabine 100 microns/ml. 2 ml Capecitabine from these solutions were drawn in 10 ml volumetric bottle with the following dimensions: 0 h, 1 h, 2 h, and 4 h and made up to the volume with the mobile phase and injected. Stress specimens deteriorated from the highest areas in the chromatogram. The damaged substance content was determined by percentage. **Table 17-18** shows the results. 0.3% H_2O_2 oxidative stress tests have been performed at room temperature.

Thermal Degradation Studies: Thermal degradation studies for both drug solutions were conducted by exposing the sample at 60 °C for 0 h, 1 h, 2 h and 4 h in hot air oven.

10 mg of capecitabine was measured into a different 10 ml bottle; the medicine was dissolved with a few ml of water and then diluted with air until the mark was over. Solution of 1000 µg/ml. 1 ml of capecitabine has been drawn from the above solutions into a 10 ml volumetric flask, up to a volume of water to achieve a final conc. 100 µg per liter. This solution was exposed to 60 °C and 2ml capecitabine was drawn to the final volume at 0 h, 1 h, 2 h and 4 h. Stress specimens suffered from the highest areas in the chromatogram, determined the percentage level of the damaged drug. **Table 19-20** shows the findings. The results are shown.

Photolytic Degradation Studies: Photo stability degradation studies of capecitabine solution was carried out in a photostability chamber by exposing to UV light. Weighed 10 mg of capecitabine into 10 ml flasks and dissolved it with only a few ml liquid and diluted it with water to get eventually conc. of 1000 µg/ml. 1 ml of capecitabine has been drawn from the above solutions to 10 ml volumetric bottle and composed up to the volume of water to finish conc. 100µg/ml.

The solutions were drawn up and pumped into the last volume at 0 h, 1 h, 2 h and 4 h in the stability chamber with 2 ml capecitabine. Stress specimens were removed from the peak areas in the chromatogram. The deteriorated drug percentage was identified **Table 21-22** show the results. The results are tabled.

RESULTS:

Validation of Analytical Method for the Assay of Capecitabine:

TABLE 2: OPTIMIZED CHROMATOGRAPHIC CONDITIONS OF CAPECITABINE

S. no.	Parameter	Result
1	Mobile Phase	MeOH : Water
2	Ratio	60: 40
3	Detector	PDA
4	Detection Wavelength	239nm
5	Column (Stationary Phase)	C18 250 × 4.5mm id, 5µm
6	Flow Rate	1ml/min
7	Column Temp.	23 °C
8	Retention time	7.927
9	Volume of injection (µL)	20

Specificity: Specificity is the ability of a method to discriminate between the intended analyte(s) and other components in the sample. Specificity of the HPLC method is demonstrated by the separation of the analytes from other potential components such as impurities, degradants, or excipients. Volume of 20 µL of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below. No peaks were found at retention time of 7.927. As no peaks were found at retention time of 7.927, the proposed method was specific for the detection of capecitabine.

Linearity and Range:

TABLE 3: LINEARITY RANGE DATA FOR CAPECITABINE

S. no.	Concentration (µg/ml)	Peak area Mean ± SD (n=3)	%RSD
1	4	612770 ± 6824.3	1.1136
2	8	906752 ± 8639.7	0.9528
3	12	1128061 ± 8824.2	0.7822
4	16	1406413 ± 8039.8	0.5715
5	20	1625569 ± 17324.6	1.0657
6	24	1905515 ± 19876.3	1.0435
7	28	2232432 ± 19763.9	0.8852
8	32	2478652 ± 21987.7	0.8870
9	36	2705543 ± 22465.1	0.8303
10	40	2971512 ± 23363.5	0.7862
11	50	3701589 ± 27864.2	0.7527

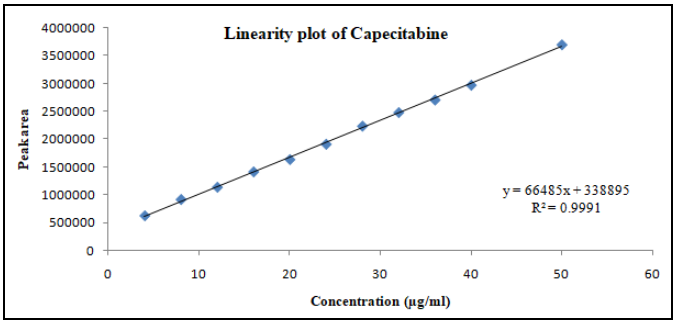


FIG. 1: LINEARITY PLOT OF CAPECITABINE

TABLE 4: LINEARITY REPORT OF CAPECITABINE

S. no.	Parameter	Values for Capecitabine
1	Linearity range	4- 50 µg/ml
2	Regression equation	Y= 66485x+ 338895
3	Correlation coefficient	0.9991
4	Intercept	338895
5	Slope	66485

Assay:

TABLE 5: ASSAY OF CAPECITABINE FORMULATION

S. no.	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3)	Assay	%RSD
1	Capiibine	150mg	152.15mg	101.43%	0.657

Accuracy:

TABLE 6: RECOVERY REPORT OF CAPECITABINE

QC conc. (µg/ml) (A)	Recovery level	Added drug (µg/ml) (B)	Total amount of drug (µg/ml) (A+B)	Peak Area Mean ± SD (n=3)	Amount found (µg/ml)	% recovery	%RSD
20	50	10	30	2382957 ± 25864	30.4347	101.4490	1.0853
	100	20	40	3069341 ± 33847	40.6754	101.6135	1.1028
	150	30	50	3600747 ± 50958	49.0614	98.1229	1.3406

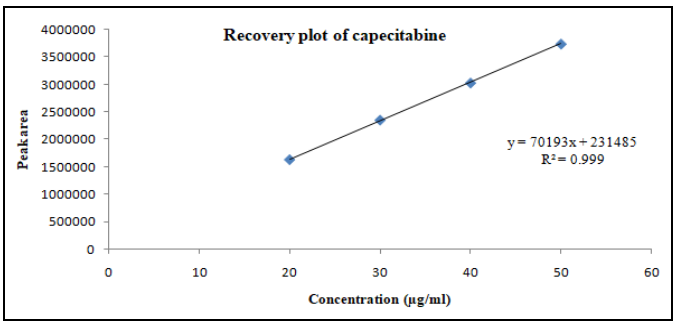


FIG. 2: RECOVERY PLOT OF CAPACETABINE

Precision:

TABLE 7: SYSTEM PRECISION DATA

Drug	Conc. (µg/ml)	Peak area mean ± S.D (n=6)	%RSD
Capecitabine	20	1648537 ± 16834.5	1.0212

TABLE 8: INTRA AND INTER-DAY PRECISION OF CAPECITABINE

S. no.	Conc. (µg/ml)	Intraday(n=3) Mean ±SD	Intraday %RSD	Interday (n=3) Mean ±SD	Interday %RSD
1	LQC(8)	953587.7±10208.67	1.070	1619319 ± 10066.45	0.621
2	MQC(20)	1864129 ±15363.69	0.824	1732015 ± 15275.25	0.881
3	HQC(40)	3742676± 58890	1.573	1552784 ± 14422.21	0.928

Robustness:

TABLE 9: ROBUSTNESS STUDIES OF CAPECITABINE

S. no.	Parameter	Optimized parameter	Modification	Retention time	Asymmetry
1	Flow rate	1ml	0.8	10.320	1.20
			1.2	6.853	1.15
2	Mobile phase composition	60:40	58:42	9.167	0.95
			62:38	7.213	1.19
3	Wave length	239nm	237nm	7.967	1.16
			241nm	7.967	1.16

Limit of Detection (LOD) and Limit of Quantification (LOQ)

TABLE 10: LOD AND LOQ REPORT OF DRUGS

S. no.	Drug	LOD	LOQ
1	Capecitabine (20µg/ml)	1.477 µ/ml	4.470 µ/ml

System Suitability:

TABLE 11: SYSTEM SUITABILITY DATA OF CAPECITABINE

Drug	Conc. (µg/ml)	Retenti-on time	Peak area (n=3) Mean ± SD	Theoretical plates
Capecitabine	20	7.927	3075298 ± 16800.89	6814

TABLE 12: SYSTEM SUITABILITY PARAMETERS FOR CAPECITABINE

S. no.	Parameter	Values obtained	Acceptance criteria
1	Retention time	7.927	---
2	Theoretical plates	6814	>2000
3	Peak area	3085506	---
4	Peak Asymmetry	1.04	≤2
5	% RSD	0.54	≤2

Stress Degradation Studies for Capecitabine:
Degradation Behavior of Capecitabine:
Degradation in Acid:

TABLE 13: ACIDIC DEGRADATION OF CAPECITABINE AT ROOM TEMPERATURE

S. no.	Time (h)	Peak area(n=3) Mean ± SD	% Degradation
1	0	1808995 ±5247.992	---
2	2	1579488 ±12222.44	12.50
3	4	1234106 ±10987.96	31.52

TABLE 14: DEGRADANTS FORMED DURING ACIDIC DEGRADATION AT ROOM TEMPERATURE

S. no.	Degradants	Retention time	Peak area (n=3) Mean ± SD
1	D_1	2.90	70137.67±247.0432
2	D_2	3.14	36151.33±146.5515
3	D_3	3.61	13732.02±116.1551
4	D_4	5.22	18680.67±111.0195

Discussion: The drug showed liability to acid hydrolysis at room temperature. It decomposed to the extent of 12.50% degradation in 0.1N HCl in 2 h. The degradation increased to 31.52% in 4 h.

Degradation in Base:

TABLE 15: ALKALI DEGRADATION OF CAPECITABINE AT ROOM TEMPERATURE

S. no.	Time (h)	Peak area(n=3) Mean ± SD	% Degradation
1	0	2098438±11516.52	---
2	2	1717719±10958.53	18.05
3	4	1406143±9729.189	32.92

TABLE 16: DEGRADANTS FORMED DURING ALKALI DEGRADATION AT ROOM TEMPERATURE

S. no.	Degradants	Retention time	Peak area Mean ± SD
1	D_1	2.507	6674.02±77.8267
2	D_2	4.787	9894.33±42.8291
3	D_3	6.487	6512.06±25.5342

Discussion: The drug showed liability to alkali hydrolysis at room temperature. It decomposed to the extent of 18.05% degradation in 0.1N NaOH in 2 h. The degradation increased to 32.92% in 4 h.

Oxidative Stress Study:

TABLE 17: OXIDATIVE DEGRADATION OF CAPECITABINE AT ROOM TEMPERATURE

S. no.	Time (h)	Peak area(n=3) Mean ± SD	% Degradation
1	0	2978072±11249.3	---
2	2	2261113±10603.21	24.02
3	4	1892712±11079.36	36.36

TABLE 18: DEGRADANTS FORMED DURING OXI-DATIVE DEGRADATION AT ROOM TEMPERATURE

S. no.	Degradants	Retention time	Peak area Mean ± SD
1	D_1	2.687	13974.33±73.6636
2	D_2	2.893	7010.02±38.9358
3	D_3	3.020	381943.7±1844.24

The drug showed highly liable to H_2O_2 at room temperature when compared to acid and alkali hydrolysis. It decomposed to the extent of 24.02% degradation in 0.3% H_2O_2 in 2 h. The degradation increased to 36.36% in 4 h.

Thermal Stress Study:

TABLE 19: THERMAL DEGRADATION OF CAPECITABINE SOLUTION AT NMT 60 °C

S. no.	Time (h)	Peak area(n=3) Mean ± SD	% Degradation
1	0	2042493 ±11276.63	---
2	2	1538681 ±11409.74	24.60
3	4	1067370 ±11507.09	42.41

TABLE 20: DEGRADANTS FORMED DURING THERMAL DEGRADATION OF SOLUTION AT NMT 60 °C

S. no.	Degradants	Retention time	Peak area (n=3) Mean ± SD
1	D_1	3.187	19029.02±73.2598
2	D_2	4.400	11989.67±21.7332
3	D_3	6.047	11936.04±74.0810
4	D_4	10.873	6227.33±70.0380

Discussion: The drug in solution form highly liable to thermal at temperature NMT 60 °C when compared to acid, alkali hydrolysis, and H_2O_2. The solution decomposed to the extent of 24.60% degradation in thermal in 2 h. The degradation increased to 42.41% in 4 h.

Photodegradation:

TABLE 21: PHOTODEGRADATION OF CAPECITABINE SOLUTION

S. no.	Time (hr)	Peak area(n=3) Mean ± SD	% Degradation
1	0	8403780±9701.546	---
2	2	6025092±26410.42	28.10
3	4	3566527±36488.95	57.24

TABLE 22: DEGRADANTS FORMED DURING PHOTO DEGRADATION OF CAPECITABINE SOLUTION

S. no.	Degradants	Retention time	Peak area (n=3) Mean ± SD
1	D_1	4.367	3431.66±30.9892
2	D_2	4.820	2085.33±29.14332
3	D_3	6.060	1046.00±12.00

The drug showed higher liability to photodegradation at room temperature. It decomposed to the extent of 28.10% degradation in 2 h. The degradation increased to 57.24% in 4 h.

SUMMARY: In the present study, the API's namely capecitabine (anticancer) the essential therapeutic agent in the treatment of cancer. Among the analytical techniques available in estimation and quantification HPLC method is an emerging technique reliable in vast areas of research that includes the other to undertake method development and validation as per ICH guidelines for the same.

In present work, forced degradation HPLC method has been developed for the estimation of capecitabine in marketed tablet formulation. Forced degradation The HPLC method was developed with the mobile phase system of MeOH: H_2O in the ratio of 60:40 v/v pH adjusted to 3 with orthophosphoric acid. The flow rate of 1ml/min was used on a C_{18} column (250 × 4.6 mm, 5µm particle size). The retention time of capecitabine was observed at 6.97 min **Table 23-24**.

TABLE 23: SUMMARY OF VALIDATION PARAMETERS

Parameter		For Capecitabine	Acceptance
Retention time		7.927	----
LOD (µg/ml)	Visualization method	1.477µ/ml	----
LOQ (µg/ml)	Visualization method	4.470µ/ml	----
Linearity	(µg/ml)	4-40	R^2 =0.9991
Accuracy	20mcg/ml	101.61%	98 – 102%
Precision (%RSD)	Inter-day	0.62%	
	Intra-day	0.82%	<2%
Assay	20µg/ml	101.43%	----
Robustness Flow rate	0.8ml	10.32	Robust
	1.2ml	6.85	
Robustness Mobile phase	58:42	9.16	Robust
	62:38	7.21	
Robustness wavelength	237nm	7.96	Robust
	241nm	7.96	
System Suitability	Theoretical plates	6814	>2000
	% RSD	0.54	<2%
	Asymmetry	1.19	0.9 – 1.2

TABLE 24: SUMMARY OF FORCED DEGRADATION STUDIES REPORT

S. no.	Condition	Exposure	Result	No. of degradants found
1	Acidic degradation	0.1N HCl at room temperature	Degraded	4
2	Alkali degradation	0.1N NaOH at room temperature	Degraded	3
3	Oxidative degradation	0.3% H_2O_2 at room temperature	Degraded	3
4	Thermal degradation	Drug solution at NMT 60 °C	Degraded	4
5	Photodegradation	Drug solution	Degraded	3

The developed and validated stability indication HPLC method is found be linear, accurate, precise, specific, and robust. Hence the method can be used routinely for estimation of capecitabine in formulations.

CONCLUSION: The present work was aimed at developing new validated RP-HPLC method for selected API and application to stability-indicating assay. The work was aimed comparatively to the earlier literature report in connection to the priority of the present investigation with respect to the ICH guideline parameters. In the present study, the API´s namely capecitabine (anticancer) an essential therapeutic agent in the treatment of cancers.

Among the analytical techniques available in estimation and quantification HPLC method is an emerging technique reliable in vast areas of research that indicated the author to undertake method development and validation as per ICH guidelines for the same.

The present work developed the RP-HPLC method for capecitabine by standard external method using MeOH: H_2O (pH was adjusted to 3 with ortho-phosphoric acid) (60:40 v/v) as mobile phase and detection was performed at 239 nm with a retention time of 7.927 min and peak asymmetry of 1.04.

The method was validated for all validation parameters as per ICH guidelines. The linearity range for capecitabine was 4-50µg/ml, with r^2 value of 0.9991. The % RSD for intra and interday precision was < 2%. The method has been validated in the assay of tablet dosage forms. The accuracy of the method was validated by recovery studies and was found to significantly and under specification limits, with % recovery 99-101.5 (within the acceptable range 98-102%). The method also passed the specifications for robustness parameters.

A stability study on capecitabine was carried out, and an efficient HPLC method for quantification of capecitabine and identification of its degradation products in the bulk drug was developed and validated. The results of stress testing of the bulk drug, undertaken according to ICH guidelines, revealed that degradation products were formed under acidic, alkaline, thermal, oxidizing, and photolytic conditions.

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