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QBD APPROACH TO DEVELOP RP-HPLC METHOD FOR CAPECITABINE IN BULK AND DOSAGE FORM

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Keywords:

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ABSTRACT: A simple, specific, accurate, reliable, and precise reverse-phase high-performance liquid chromatographic method was developed by quality by design approach and validated for the estimation of Capecitabine in bulk and tablet dosage forms. A surface methodology was used to optimize the data with a three-level Box Behnken Design (BBD) was used. Mobile phase composition, flow rate, and column oven temperature were chosen as the three variables. The linearity was over the concentration range of 1-15 µg/ml with a correlation coefficient of 0.999. The LOD and LOQ were found to be 0.103 ug/mL and 0.313 ug/mL, respectively. Chromatographic separation was carried out by using a mobile phase of methanol: water (70:30 V/V) on Kromasil C18 column (250 mm X 4.6mm ID, 5 µm) in an isocratic mode at a flow rate of 0.9 ml/min with UV detection at 239 nm. The developed RP-HPLC method yielded a suitable retention time for Capecitabine of 4.70 min, which was optimized using the Design-Expert version 7.0.0. The percentage recovery was found to be 99.81%. The RSD percentage for methods precision was less than 2%. The developed methods were found to be precise and accurate for estimating Capecitabine in pharmaceutical dosage forms and could be used for routine analysis.

INTRODUCTION: Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a pro-drug that is enzymatically converted to fluorouracil (antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue [1, 2, 3]. The activation of Capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5`-deoxy-5-fluoro-cytidine (5`-DFCR) and 5`-deoxy-5-fluorouridine (5`-DFUR) to form 5-fluorouracil [4, 5]. The empirical formula of Capecitabine is $C_{15}H_{22}FN_3O_6$ and its molecular weight is 359.35 g/mol [6].

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Quality by Design (QbD) is a systematic approach integrated with quality risk management (QRM) using different statistical and experimental designing tools to produce a quality product. Implementing quality by design to analytical method has multiple advantages compared to traditional method development and optimization. These include developing a regulated and robust analytical method for application throughout the lifecycle of a pharmaceutical product [7]. Quality by design is a modern, scientific approach that formalizes product design, automates manual testing, and streamlines troubleshooting [8].

Capecitabine is a fluoropyrimidine carbamate, antimetabolite class of antineoplastic drug [9]. QbD helps build the quality of products by design through risk assessment at the early stage and defining the design space later. QbD-based product development enables the understanding of additional formulation aspects by using a scientific approach and quality risk management [10].

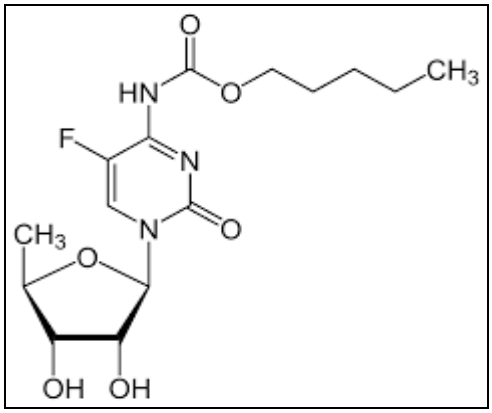

FIG. 1: MOLECULAR STRUCTURE OF CAPECITABINE

MATERIAL AND METHOD:

Materials and Reagents: Methanol was provided by Qualigen (Thermo fisher scientific). Moreshwar Enterprises provided HPLC Water.

Instrumentation and Software: An Agilent HPLC system with DEAX02386 pump and autosampler with UV-visible detector served as the chromatographic system (DEACX16446). For data collection and processing, the chromatograms were registered using Openlab EZChrome workstation on a Windows-based computer system. Qbd software- Design Expert® software (Design-Expert version 7.0.0; State-Ease Inc., Minneapolis, MN, USA).

Chromatographic Condition:

TABLE 1: CHROMATOGRAPHIC CONDITIONS

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	239 nm
Column Oven temp	38ºC
Mobile Phase	Methanol : Water (70:30%V/V)
Flow Rate	0.9 ml/min
Run time	10 Minutes

Preparation of Standard Solutions for UV Scan to Determine Absorption Maxima Wavelength: In order to prepare a stock solution, weighed accurately 10 mg Capecitabine and transferred into 50 ml volumetric flask, added 35 ml of water and sonicated to dissolve the standard completely and diluted up to the mark with water (200 PPM). Further diluted 2 mL to 20 mL with water. (20 PPM).

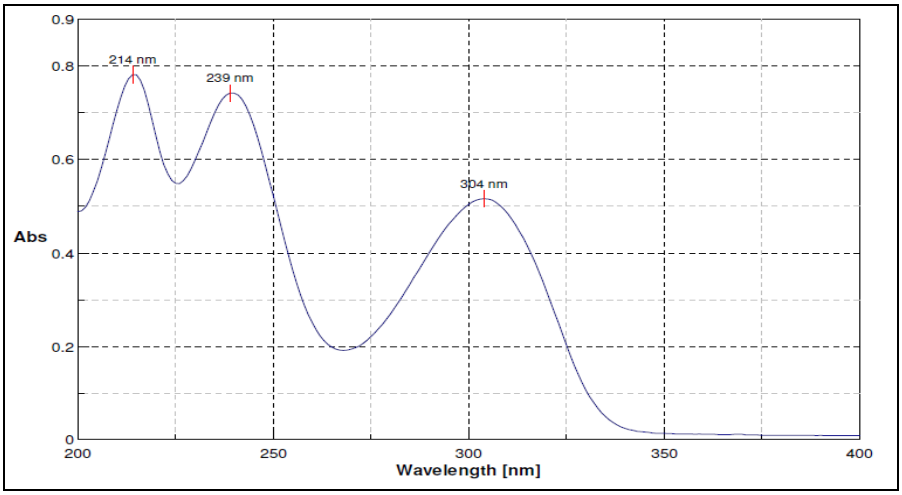

FIG. 2: UV SPECTRUM OF CAPECITABINE

Method Development by RP-HPLC:

Preparation of Standard Solution for Chromatographic Development: Capecitabine Standard stock solution was prepared by dissolving 10 mg Capecitabine into a 20 mL clean and dried volumetric flask, adding about 15 mL of water to dissolve it completely and making the volume up to the mark with water. (500 PPM). Further diluted 2 ml of stock solution to 10 mL with water. (100 PPM).

Optimization of Developed RP-HPLC Method with Design Space and Control Strategy Determination by Optimization study:

Application of Design of Experiments for Method Optimization: Thus, 3^3 randomized response surface designs with a Box-Behnken design were used with 17 trial runs to study the impact of three factors on the three key response variables. In this design, 3 factors were evaluated, each at 3 levels, and experimental trials was

performed at all 3 possible combinations. The mobile phase composition (X1), flow rate (X2) and column oven temperature (X3) were selected as independent variables, and retention time (RT), asymmetry, and theoretical plates were selected as dependent variables. The resulting data were fitted into Design Expert 7.0.0. Software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to response surface methodology to determine the influence of mobile phase composition, flow rate, and column oven temperature on dependent variables. The probable trial runs using 3^3 Box Behnken designs are shown in **Table 2.**

TABLE 2: THE LAYOUT OF THE ACTUAL DESIGN OF DOE

Runs	Factor1	Factor 2	Factor3	Response 1	Response 2	Response 3
	A: % Methanol	B: Flow rate	C: COT (°C)	Retention time (RT)	Asymmetry	TP
1	60	0.9	35	7.30	1.11	10721
2	60	1.0	32	6.73	1.12	8695
3	70	1.0	35	4.27	1.17	8772
4	70	0.9	32	4.85	1.15	9110
5	70	1.0	35	4.27	1.17	8781
6	80	1.0	32	3.30	1.13	6682
7	70	1.1	32	3.95	1.15	7791
8	60	1.0	38	6.47	1.11	10768
9	70	1.0	35	4.27	1.18	8750
10	70	1.1	38	3.83	1.14	9324
11	70	0.9	38	4.70	1.12	10718
12	70	1.0	35	4.28	1.18	8758
13	80	0.9	35	3.65	1.14	7534
14	80	1.0	38	3.24	1.13	8900
15	70	1.0	35	4.27	1.17	8786
16	60	1.1	35	5.93	1.11	9179
17	80	1.1	35	2.98	1.13	6652

Validation of RP-HPLC Method: The optimized method for estimation of Capecitabine was validated as per ICH guidelines for the following parameters.

1. Filtration Study: A filtration study of an analytical procedure checks the interference of extraneous components from the filter, deposition on the filter bed, and filter compatibility with the sample.

Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF was used by discarding 5 mL of aliquot sample.

2. Stability of Analytical Solution: A stability study was conducted for standard and test sample solutions. A stability study was performed at normal laboratory conditions.

The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours. Standard and Test solution stability study was performed by calculating the difference between the results of the test solution at each stability time point to that of the initial.

3. Specificity: Specificity is the ability to access the analyte unequivocally in the presence of components that may be expected to be present. The following solution shall be prepared and injected to prove the specific nature of the method.

- Blank (Water as a diluent).
- Placebo.
- Capecitabine Standard solution.
- Tablet test sample solution.

4. Linearity and Range: 5 levels of linearity were performed from 10% to 150% of working concentration. Each level was injected in triplicate, and the mean area was calculated. The calibration curve was plotted graphically as a function of analyte concentration in µg/mL on the X-axis Vs the mean area on y-Axis as given in the results.

5. Limit of Detection (LOD) and Limit of Quantitation (LOQ): As per ICH Q2R1 guidelines, LOD and LOQ were determined by using the approach based on the Calibration Curve in which the residual standard deviation of a

regression line was calculated, and determined the LOD and LOQ by using the following formula:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where, σ = residual standard deviation of a regression line, S = Slope of the regression line.

6. Accuracy (% Recovery): Accuracy will be conducted in the range from 50 % to 150 % of working concentration.

The solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery, and % RSD for each level and % RSD for overall recovery.

7. Precision: Precision is of two types, Repeatability and Intermediate precision. It is performed on the tablet test sample.

8. Robustness: Blank and Standard solutions were injected under different chromatographic conditions as shown below.

A. Changes in flow rate by ±10%. (± 0.09ml/min)
B. Change in column oven temperature. (± 2ºC)
C. Change in wavelength (± 3 nm)

RESULT:

Optimization of Mobile Phase: Trial no. 11 was selected as an optimized chromatography, as it has Optimum R., Good asymmetry, and theoretical plates. Typical chromatogram of the optimized method given in **Fig. 3.**

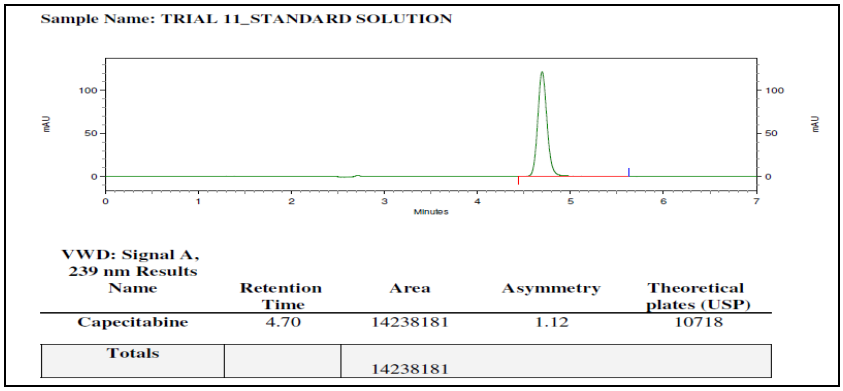

FIG. 3: TYPICAL CHROMATOGRAM OF OPTIMIZED METHOD

System Suitability Test: It was observed from the data tabulated that the method complies with system suitability parameters. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation. A typical chromatogram of SST for Capecitabine is shown in **Fig. 4**. Analytical data of the system suitability test are given in **Table 3.**

TABLE 3: ANALYTICAL DATA OF SYSTEM SUITABILITY TEST

Parameter	Acceptance criteria	Result
%RSD	NMT 2.0%.	0.13
Theoretical plates	More than 2000	10338
Tailing factor	NMT 2.0	1.13

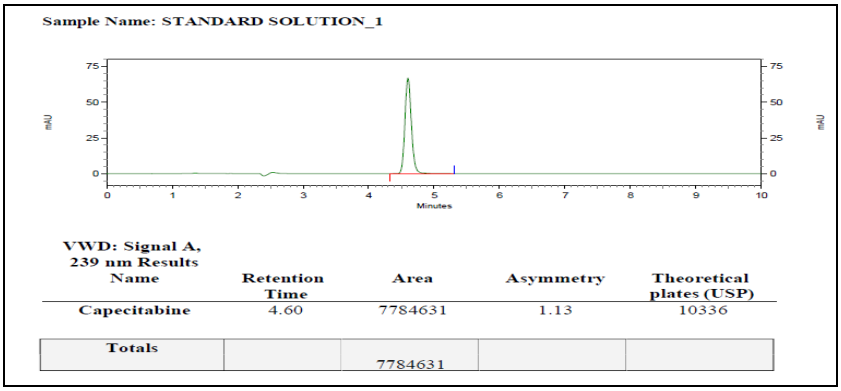

FIG. 4: TYPICAL CHROMATOGRAM OF SYSTEM SUITABILITY SOLUTION FOR CAPECITABINE

Filter test: Filters PVDF pass the criteria for filter study; hence, the filter can be used because % the absolute difference is NMT 2.0, and it follows acceptance criteria. Analytical data of the filter test are given in **Table 4**. A typical chromatogram of the unfiltered sample, a sample filtered through a 0.45 µ PVDF filter, is shown in **Fig. 5, 6**.

TABLE 4: ANALYTICAL DATA OF FILTER TEST

Sample description	Area	% Absolute difference
Unfiltered	7571413	NA
0.45 µ PVDF filter	7559741	0.15

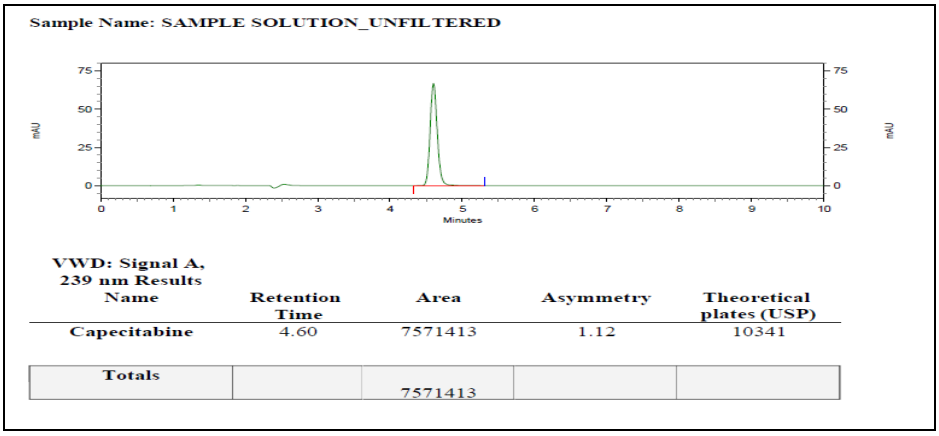

FIG. 5: TYPICAL CHROMATOGRAM OF UNFILTERED SAMPLE

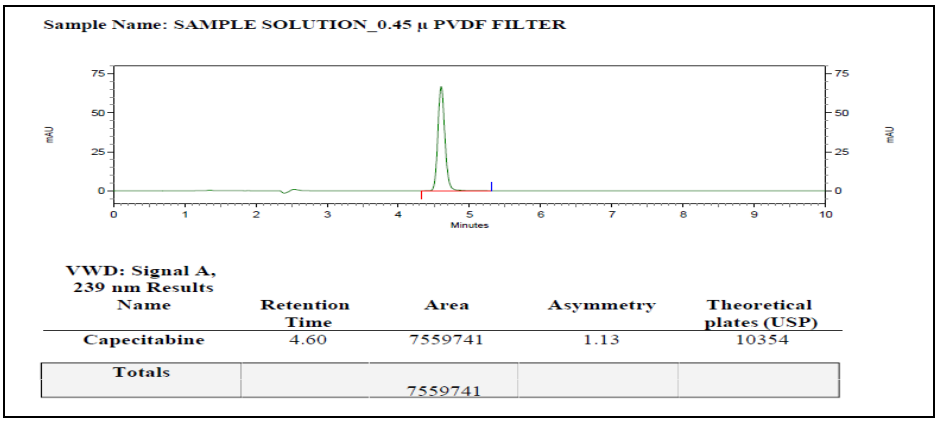

FIG. 6: TYPICAL CHROMATOGRAM OF SAMPLE FILTERED THROUGH 0.45µ PVDF FILTE

Solution Stability: Both standard solution and sample solution were found stable for 24 hrs; hence, prepared solution can be used up to 24 hrs. Analytical data are given in **Table 5.**

Acceptance Criteria: % Absolute difference of Stability solution: NMT 2.0 w.r.t. Initials.

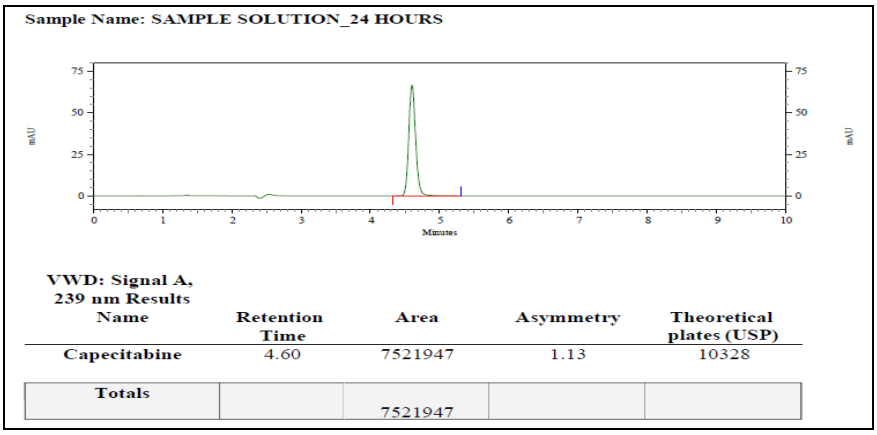

FIG. 7: TYPICAL CHROMATOGRAM OF TEST SOLUTION AFTER 24 HRS

TABLE 5: ANALYTICAL DATA OF CAPECITABINE FOR SOLUTION STABILITY

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	7575417	NA	Initial	7786417	NA
12 Hours	7564014	0.15	12 Hours	7764217	0.29
24 Hours	7521947	0.71	24 Hours	7724176	0.80

Specificity: Blank and placebo solutions do not have interference at R.T. of Capecitabine. Peak purity for both standards, as well as the sample, was within limits. The sample solution exhibits the same R.T. as that of the standard solution. Hence, the developed chromatographic method passed the criteria for specificity. The result of specificity is given in **Table 6.**

TABLE 6: RESULTS OF SPECIFICITY

Description	Observation	Acceptance criteria
Blank	No interference at R.T. of Capecitabine due to blank	No interference at R.T.
Placebo	No interference at R.T. of Capecitabine due to placebo	No interference at R.T.
Standard solution	Peak purity was 0.987	Peak purity: NLT 0.95
Test Solution	Peak purity was 0.974	Peak purity: NLT 0.95

Linearity: The calibration curve concluded that the Capecitabine shows a linear response of 1.0-15.00 µg/ml. The Regression value was found well within the limit. Linearity data of Capecitabine is given in **Table 7.** The linearity graph of Capecitabine is shown in **Fig. 8.**

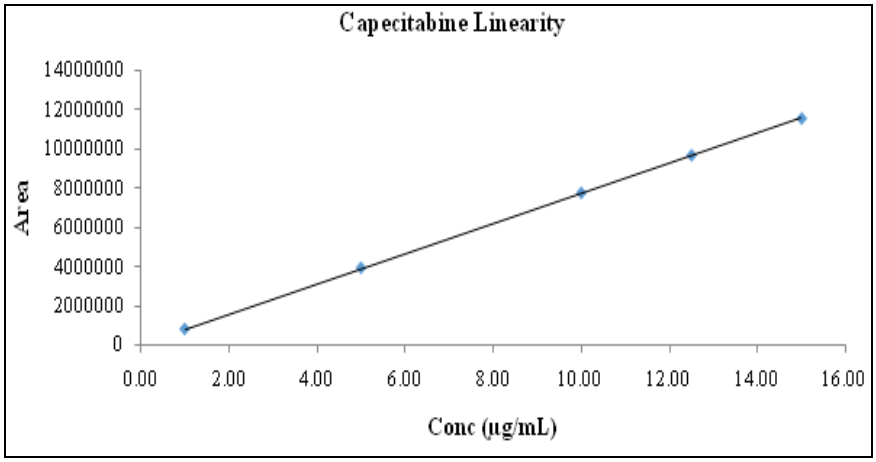

FIG. 8: LINEARITY GRAPH OF CAPECITABINE

TABLE 7: LINEARITY DATA FOR CAPECITABINE

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	1.00	804859	805467	0.079
		805419		
		806124		
50%	5.00	3922812	3928089	0.252
		3939517		
		3921937		
100%	10.00	7773717	7759911	0.177
		7746170		
		7759847		
125%	12.50	9691186	9683984	0.078
		9684617		
		9676148		
150%	15.00	11647789	11562811	0.681
		11548471		
		11492174		

Detection: It may be calculated based on the response's standard deviation (SD) and slope of the curve (S). The result of the detection limit is given in **Table 8.**

TABLE 8: RESULT OF DETECTION LIMIT

Parameter	Result
LOD	0.103
LOQ	0.313

Accuracy (%recovery): %Recovery was found well within the acceptance range (98.00 to 102.0%) at all three levels. Results and statistical data of accuracy are given in **Table 9.**

TABLE 9: RESULT AND STATISTICAL DATA OF ACCURACY OF CAPECITABINE

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD	Overall % Recovery	Overall% RSD
50	3849637	5.03	5.02	100.20	99.47	0.7061	99.81	1.054
	3811921	4.98	5.01	99.40				
	3786175	4.95	5.01	98.80				
100	7760187	10.15	10.02	101.30	100.53	0.7466		
	7721087	10.09	10.04	100.50				
	7641804	9.99	10.01	99.80				
150	11284198	14.75	15.04	98.07	99.42	1.4863		
	11386304	14.89	15.01	99.20				
	11596386	15.16	15.01	101.00				

Precision: %RSD for 12 samples (precision and intermediate precision samples) NMT 2.0%. The %RSD of method precision is 1.242 and 0.985. Therefore, the HPLC method for the determination of Capecitabine is precise. Analytical data of both precisions of Capecitabine is given in **Table 10.**

TABLE 10: DATA OF PRECISION OF CAPECITABINE

Parameters	Intraday precision	Interday precision	Acceptance criteria
Mean	98.57	98.60	90-110 %
SD	1.2242	0.971420	
% RSD	1.242	0.985	% RSD for the six samples NMT 2.0

Robustness: An analytical method's robustness is determined by analyzing aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. Analytical interpretation is given in **Table 11.**

TABLE 11: RESULT OF ROBUSTNESS STUDY

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (242 NM)	4.60	7777210	1.13	9938
Wavelength by -3 NM (236 NM)	4.60	7355051	1.16	9231
Flow rate by +10% (9.90 mL/min)	4.16	7040155	1.15	8202
Flow rate by -10% (0.81 mL/min)	5.16	8722186	1.17	10544
Column oven temp by +2ºC (40 ºC)	4.58	7784205	1.15	8650
Column oven temp by -2ºC (36 ºC)	4.58	7706447	1.18	8427

CONCLUSION: All the validated parameters were found within the acceptance criteria. The validated method was found to be linear, precise, accurate, specific, and robust for determining Capecitabine. The QbD approach to method development has helped understand the method variables better, leading to less chance of failure during method validation and transfer. This method will be used further for routine pharmaceutical industry quality control analysis.

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