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RP- HPLC method development and validation of Capecitabine in bulk form by using QbD.

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Abstract

The simple, rapid spectrophotometric method was developed for the determination of capecitabine, an anticancer drug, in pharmaceutical formulations. The focus of the present study is to similarize the capecitabine drug in bulk form by simple, accurate, and precise manner by UV spectrophotometer. Beer's law was obeyed over a concentration range of 10–60 µg/mL in phosphate buffer at pH 6.8. The linear regression equations of pure drug were found to be linear. The developed was validated as per the International Council for Harmonisation guideline (ICH). The regression values of every equation were found to be above 0.990 which indicated that all the equations were maintaining linearity.

Keywords: RP- HPLC, Capecitabine, QbD.

INTRODUCTION

A QbD is defined as "A systemic approach to the method development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [1]." The QbD approach emphasizes product and process understanding with quality risk management and controls, resulting in higher assurance of product quality, regulatory flexibility, and continual improvement. The QbD method was based on the understanding and implementation of guidelines ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System [2–4]. Analytical science is considered to be an integral part of pharmaceutical product development and hence go simultaneously during the entire product life cycle. Analytical QbD defined as a science and risk based paradigm for analytical method development, endeavoring for understanding the predefined objectives to control the critical method variables affecting the critical method attributes to achieve enhanced method performance, high robustness, ruggedness, and flexibility for continual improvement [5, 6]. The result of analytical QbD is well known, fit for purpose, and robust method that reliably delivers the intended output over its lifecycle, similar to the process QbD [7, 8]. For QbD, HPLC methods, robustness, and ruggedness should be tested earlier in the development stage of the method to ensure the efficiency of the method over the lifetime of the product [9]. Otherwise, it can take considerable time and energy to redevelop, revalidate, and retransfer analytical methods if a non-robust or non-rugged system is adapted. The major objective of QbD has been to identify failure modes and establish robust method operable design region or design space within meaningful system suitability criteria and continuous life cycle management. Literature survey reveals QbD approaches for HPLC method were reported [10].

Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. [11-12] 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. 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. The empirical formula of Capecitabine is C15H22FN3O6 and its molecular weight is 359.35 g/mol. Capecitabine is a fluoropyrimidine carbamate, antimetabolite class of antineoplastic drug. 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. [13]

MATERIALS AND METHODS [10-12]

Capecitabine was procured as a gift sample from Cipla Ltd. All other the Chemicals used for method development are of HPLC grade includes Acetonitrile, water, methanol were purchased from Merck (India) Ltd.

Methods

Identification of API

A. Capecitabine

1. Organoleptic Characteristics

It is a white to pale yellow crystalline, non-hygroscopic powder.

2. Solubility-

The solubility of Capecitabine was determined. 10mg drug was dissolved in water and ethanol. Depending on the visual observation for drug particles in the solvents, the results were obtained.

3. Melting point determination

It was determined by melting point apparatus (Superfit, India) after filling the drug powder in capillary tubes (heat-sealed at one end).

4. Differential Scanning Calorimetry (DSC) of Capecitabine

Approximately 10mg of Capecitabine was weighed and placed in the crucible. The crucible was sealed using small lid and crimped and analyzed at temperature range between 30- 400˚C at heating rate 10˚C per minute. Then the sample was placed in the DSC apparatus and endothermic or exothermic nature of the drug substance was analyzed.

5. Infrared spectroscopy of Capecitabine

IR spectra of the drug was obtained by scanning in the range between 400 to 4000 cm-1. The interpretation was seen form literature available. The spectrum was recorded and compared with standard spectrum.

5. UV Spectroscopy: (Determination of λ maximum)

Linearity shall be established by demonstrating that the absorbance obtained is directly proportional to the concentration of the standard solution. The standard solutions are to be prepared at 6 different concentration levels ranging of working concentration and finding the response at each concentration level for assay.

Procedure

Accurately weighed 50mg of Capecitabine was transferred into a clean and dry 50ml volumetric flask, a minimum required volume of Phosphate buffer (pH 6.8) was added, the volumetric flask was shaken gently to dissolve whole amount of the drug and the volume was made up to 100 ml with Phosphate buffer (pH 6.8) to obtain 100µg/ml stock solution. The aliquots 0.5 ml, 1.0 ml, 1.5 ml, 2.0, 2.5, 3.0, 3.5,4.0, 4.5, 5.0 ml were taken in 10 ml volumetric flasks and volumes were made up to the mark with Phosphate buffer (pH 6.8). The resulting concentrations ranged from 5-50 µg/ml. The absorbance of each concentration was determined at 301nm in a UV-Visible spectrophotometer (Jasco-V630) against Phosphate buffer (pH 6.8) as blank. The standard curve was prepared between absorbance and concentration.

Instrumentation

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column.

Chromatographic conditions

An Jasco gradient HPLC system with manual injector and UV was used for the purpose of separation. The separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column.
by using mobile phase vary in the ratio for the development of the HPLC method.

Determination of Lambda maximum

Preparation of stock solution of Capecitabine

Capecitabine (50 mg) in a 25mL volumetric flask and 25 mL of Phosphate buffer (pH 6.8) to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking Phosphate buffer (pH 6.8) as blank. The lambda maximum for Capecitabine was found to be 301nm.

Preparation of mobile phase
The preparation of mobile phase was done by mixing Phosphate buffer (pH 6.8) with Acetonitrile in the ratio of 85:15. Removal of gases was carried out in ultrasonic Acetonitrile bath for 15 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation
Mobile phase used as diluents.

Preparation of standard stock solution
50mg of Capecitabine standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution
50mg equivalent of Capecitabine pure drug was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength
It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Capecitabine was carried out under UV ranging from 200-400nm using the standard solution.

Methodology
The optimization of chromatographic conditions was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column. The separation was done by utilizing Phosphate buffer (pH 6.8): Acetonitrile 80:20% v/v ratio, Phosphate buffer (pH 6.8): Acetonitrile 85:15% v/v ratio and Acetonitrile: Methanol 80:20% v/v ratio the volume of sample was 20µl. The flow rate was maintained at 1.5ml/min. The detection of drug Capecitabine was done at 301nm.

DESIGN OF EXPERIMENT
Central Composite designs by Design expert 13 Software
When the factor number is greater than 2, the number of experiments required for this design (calculated by expression N = 3k, where N is the experiment number and k is the factor number) is very large, reducing its efficiency in the modelling of quadratic functions. Because a complete Central Composite design with more than two variables necessitates more experimental runs than are typically available in practise, designs with a smaller number of experimental points are preferred. The vast majority of Central Composite factorial designs are used in chromatograph
Following mobile phases selected
Phosphate buffer: Acetonitrile
Acetonitrile: Methanol
Central Composite Factorial design facilitate only one mobile phase at a time
Phosphate buffer: Acetonitrile
Acetonitrile: Methanol
Change pH Range:6.8-7.2
Change Mobile phase proportion Range: 80-20%
When all of the above ranges were combined in a Central Composite design, it resulted in 13 runs with varying pH and mobile phase proportions.
The same procedure was followed for each mobile phase, as column C-18 has two mobile phases. The total number of design runs is 28. After all trails have been completed, the software will provide the best value for the given chromatographic conditions. By maximizing desired factors and minimizing undesired ones, optimization means finding an alternative with the most cost effective or highest achievable performance under the given constraints. In contrast, maximization refers to attempting to achieve the highest or most favorable result or outcome without regard for cost or expense.
For method development, a central composite design was used to assess the effects of buffer amount, buffer pH, and flow rate on responses. The software recommended a total of 14 runs. The table shows the factors and responses that were considered for the study. Previous univariate chromatographic separation studies were used to determine the ranges to be considered. The organic amount ranged from 10 to 20% v/v, the pH of the buffer was 6.8 to7.2, and the flow rate was 1.5mL min.

Table 1: Total 14 runs were suggested by the software

Sr. No	Mobile Phase Composition (Aqueous Phase)	pH of Buffer
1	85.00	6.8

2	80.00	6.8
3	95.00	7.0
4	80.00	6.8
5	80.00	7.2
6	95.00	6.8
7	80.00	6.8
8	40.00	6.5
9	95.00	6.5
10	80.00	6.8
11	80.00	6.8
12	80.00	6.8
13	95.00	6.8
14	80.00	7.2

3. Preparation of mobile phase

1. 85 mL of Phosphate buffer (pH 6.8) were combined with 15 mL of ACN.
2. The solution was filtered through a 0.45 membrane filter and then sonicated for 10 minutes in a sonicator bath.

4. Preparation of stock solutions of Capecitabine

1. Stock solution was made by dissolving 10 mg Capecitabine in water and then diluting it with water in a volumetric flask of 10 ml to achieve a concentration of 1000 g/ml.

2. 0.1 ml of the resulting solution was diluted to 10 ml with water to obtain a concentration of 10 g/ml of Capecitabine, which was labelled as standard stock.

5. Selection of detection wavelength

Further dilutions of the standard stock solution were made with water and scanned over the range of 200-400 nm, with the spectra being overlain. It was discovered that the drug had a high absorbance at 301nm.

Method Validation

Linearity*:*

The linearity of the developed method was studied over the concentration ranges between 10- 60µg/ml. The obtained concentrations were injected into the chromatographic system. Calibration curve of Capecitabine was constructed by plotting peak area versus used concentration of Capecitabine. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Capecitabine, a known amount of standard drug powder of Capecitabine was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution was made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

1. Organoleptic Characteristics

Table 2- Active Pharmaceutical Drug

Sr. No.	Name	Description
1.	**Capecitabine**	Yellowish crystalline powder

2. Solubility

Capecitabine soluble characteristic by dissolving approximately 10mg/mL.
The solubility profile of Capecitabine is mentioned below in the table.

Solvent	Solubility parameter
ethanol	slightly soluble
Water	insoluble

3. Melting point determination
It was determined by melting point apparatus. The melting point was found to be 118.58°C, which is complying as mentioned in the monograph given in official pharmacopoeia.
4. Differential Scanning Calorimetry (DSC) of Capecitabine
Figure shows the DSC thermogram of pure drug Capecitabine. The endset was seen at 119.14˚C. The peak of the endotherm was similar to the melting point of the drug.

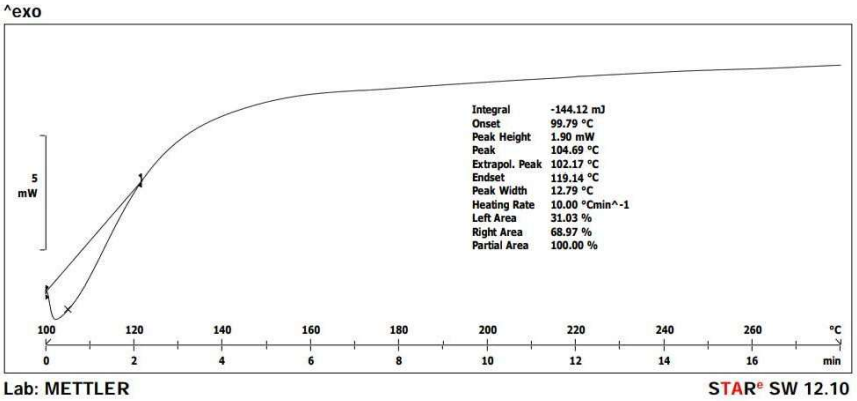

Figure 1. DSC thermogram of pure drug Capecitabine

5. Infrared spectroscopy of Capecitabine
IR spectra of the drug was obtained by scanning in the range between 400 to 400 cm-1. The interpretation was seen form literature available. The spectrum was recorded and compared with standard spectrum.

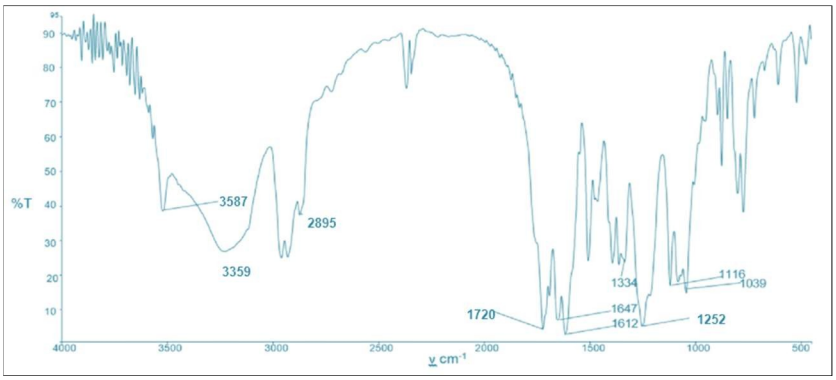

Figure 2. FTIR spectra of pure drug Capecitabine

Pure Capecitabine showed characteristic IR absorption bands at 3587, 3359, 2895, 1720, 1647, 1252 were thought to be O–H stretch, CH Stretch (alkane), CH stretch (aromatic), CH Stretch (alkene) C O stretching from amide I, N–H bending and C–N stretching from amide II,–CH bending, –CH symmetrical deformation, and skeletal vibration ofC–O stretching,
6. Linearity by UV
Linearity shall be established by demonstrating that the absorbance obtained is directly proportional to the concentration of the standard solution. The standard solutions are to be prepared at 6 different concentration levels ranging of working concentration and finding the response at each concentration level for assay.
Selection of wavelength
Accurately weighed quantity of the sample equivalent to 20 mg of Capecitabine was taken in a 20 mL volumetric flask. 12.5 mL of methanol was added, sonicated for 15 min and filtered through 0.45 µm nylon filter. The above solution

was scanned between 200 and 400 nm by UV spectroscopy.

Procedure

Record the UV absorbance spectrum of the standard and sample solution between 200 to 400nm using diluent in 1cm cell. Capecitabine showed maximum absorbance at 303nm by using methanol as diluent.

Table 3. Conc. Vs AUC.

Conc.	AUC
10	15487
20	31025
30	45874
40	60148
50	75489
60	90143

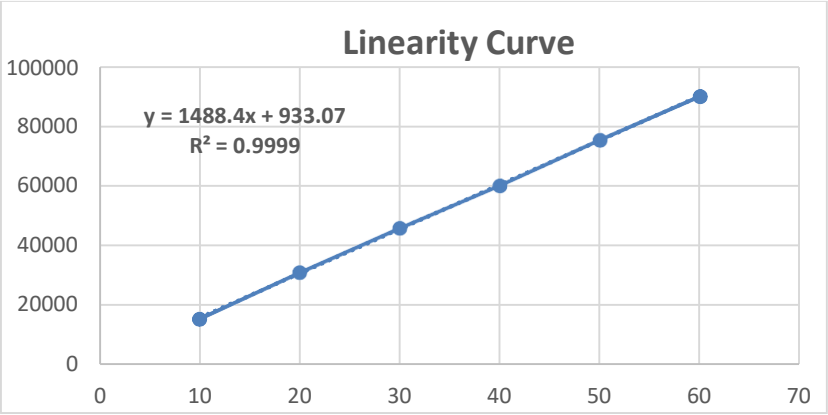

Figure 3. Linearity curve of Capecitabine

METHOD DEVELOPMENT

The proposed chromatographic method was found to be suitable for effective separation of Capecitabine with good resolution, peak shape given in the figure. The mobile phase composed of Phosphate buffer (pH 6.8): Acetonitrile 80:20% v/v ratio, at a flow rate of 1.5 ml/min was selected as it gave well resolved peaks of standard Capecitabine. The optimum wavelength 301nm selected for detection and quantitation.

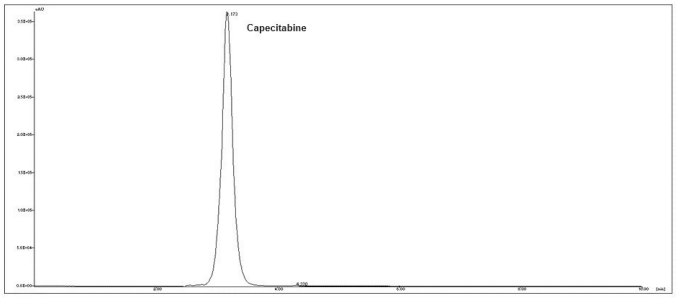

Figure 4. HPLC Chromatogram with resolved peak of Capecitabine

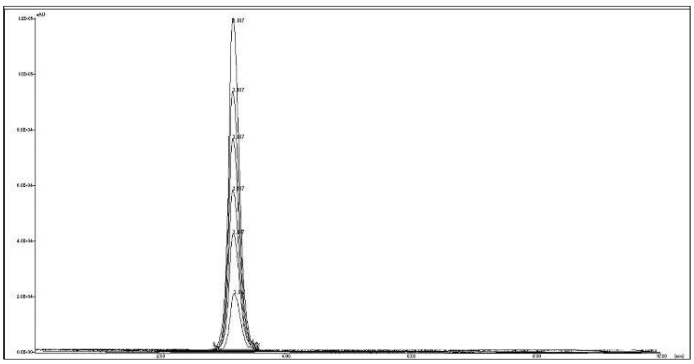

Figure 5. HPLC Linearity Chromatogram of Capecitabine

METHOD VALIDATION

Linearity

The calibration curves were found be linear for the concentration range of 10-60ppm. The standard working curve equation for drug was found to be y = 1488.4x + 933.07 with correlation coefficient value r^2 = 0.9999. The results of linearity are given in Table and Figure.

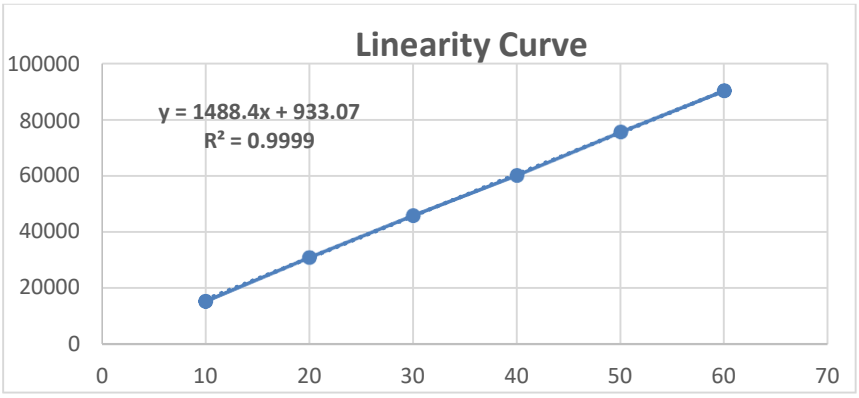

Figure 6. Linearity curve of standard Capecitabine

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Capecitabine given in Table. The % recovery at 80, 100, and 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table-4: Recovery data of Capecitabine

Level (%)	Drug Conc. (mg)	Amt. recovered (mg)	% Recovery
80	8	8.1	101.25
100	10	10.47	104.7
120	12	12.36	103

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The results of precision studies are shown in Table.

Table- 5: Precision study (intra- day) of Capecitabine

Conc µg/mL	Area	AVG	SD	%RSD
10	15485	15457.333	42.0039681	0.27174136
	15478			

	15409			
20	30258	30202.667	69.9809498	0.23170454
	30124			
	30226			
30	45789	45782	23.3023604	0.05089852
	45801			
	45756			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table- 6: Precision study (inter-day) of Capecitabine

Conc µg/mL	Area	AVG	SD	%RSD
10	15326	15310	55.7494395	0.36413742
	15248			
	15356			
20	30589	30456	268.46415	0.88148198
	30147			
	30632			
30	45002	45054	81.559794	0.18102675
	45012			
	45148			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

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

This data showed that the sensitivity of method to determine the drug Capecitabine. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 2.41 & 3.04 µg/m/ respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Capecitabine by changing flow rate 1.3 mL/min and 1.7 mL/min, mobile phase composition Phosphate Buffer: Acetonitrile ratio 84:16 and 86:14 wavelength i.e. 300nm and 302nm. The method was found to be robust in the range of deliberate changes made.

Table-7: Robustness study with change in flow rate of Capecitabine

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
1.3	20	30224	30423.33	0.58638
1.3		30478		
1.3		30568		
1.7	20	30698	30601.33	0.27427
1.7		30547		
1.7		30559		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table-8: Robustness study with change in concentration of mobile phase of Capecitabine

Mobile phase (Acetonitrile: 01% OPA)	Conc µg/mL	Area	AVG	%RSD
84:16	20	30114	30423.33	0.88299
84:16		30598		
84:16		30558		
86:14	20	30458	30530.33	0.20534
86:14		30569		
86:14		30564		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table-9: Robustness study with change in Wavelength of Capecitabine

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
300	20	30669	30627.33	0.19477
300		30654		
300		30559		
301	20	30587	30547	0.25275
301		30596		
301		30458		

CONCLUSION

As can be seen from the validation findings, the RP-HPLC test technique for Capecitabine created using the QbD methodology is linear, accurate, exact, repeatable, and specific. The created approach simply identifies stability and may be utilised for quality control to establish the test in routine Capecitabine product development, production, and stability samples. From the results of the analysis, it can be concluded that the proposed method is precise, easy, accurate, sensitive, and selective for the quantification of capecitabine from its marketed formulations. It can be used for the biopharmaceutical studies with slight modification.

CONFLICT OF INTEREST

None declared by authors.

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