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Design of Experiments (DoE) - Based Enhanced Quality by Design Approach to Hydrolytic Degradation Kinetic Study of Capecitabine by Eco-friendly Stability Indicating UV-Visible Spectrophotometry

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ABSTRACT

Capecitabine is an anti-metabolite class of anti-neoplastic drug that is converted to fluorouracil in body tissue. Numbers of stability indicating chromatography method such as HPLC, HPTLC etc. have been reported for stability study of capecitabine. But the chromatography methods are tedious, time and solvent consuming methods. Hence, stability-indicating UV-visible spectroscopy method has been developed for hydrolytic degradation kinetic study of capecitabine using design of experiments (DoE)-based enhanced quality by design approach (QbD) to save time, solvent and cost of analysis. The potential method parameters were identified and assessed by risk priority number (RPN) ranking and filtering. The DoE-based full factorial designs were applied for degradation kinetic study in alkaline and acidic medium at different temperature. The rate constant, order of reaction, half-life and % degradation of capecitabine was calculated. The absorbencies of all samples were measured at 307nm wavelength and linearity of capecitabine was found to be in the range of 5-25µg/mL. The order of reactions for alkaline and acidic degradation kinetic was found to be first order. The highest degradation rate constant and % degradation of capecitabine was found to be in 0.3 N acid or base at 50˚C. The prediction of rate constant and % degradation of capecitabine was done using DoE-based response surface analysis. The data of degradation kinetic was found to be in good agreement with published RP-HPLC method of capecitabine.

Keywords: Stability indicating UV-Visible spectrophotometry, Capecitabine, Design of experiments (DoE), Enhanced-QbD approach, Full-factorial design

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INTRODUCTION

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a prodrug that is enzymatically converted to fluorouracil (antimetabolite) in the tumour, where it inhibits DNA synthesis and slows the growth of tumour tissue. Chemically it is 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] – cytidine. It is used to treat cancer of the colorectal, breast and stomach and oesophagus [1]. The chemical structure of capecitabine is shown in figure I.

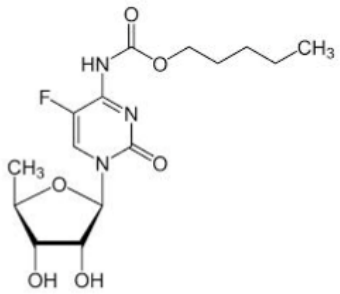

Figure I: Chemical structure of capecitabine

Degradation kinetic studies give the information regarding the rate of the process that generally leads to the inactivation of the drug through either decomposition or loss of drug by conversion to a less favourable physical or chemical form. Hence, the study of degradation kinetic is important for the development of stable formulation, determining optimum storage conditions, selecting the proper container for dispensing and predicting the shelf life of drug [2,3].

DoE-based quality by design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The concept of QbD applied to analytical method development is known as Analytical Quality by Design (AQbD). A current trend among the pharmaceutical industry is to implement AQbD as a part of risk management, and pharmaceutical quality system and pharmaceutical development [4-7].

Numbers of analytical methods such as RP-HPLC, LC-MS, UV-Visible spectrophotometric method, stability-indicating RP-HPLC methods have been reported for estimation of capecitabine [8-20]. But there was no stability-indicating UV-visible spectrophotometry method was found reported by which capecitabine can be estimated independently to degradation products formed in different stress conditions such as hydrolysis, photolysis, oxidation, dry heat degradation etc. The degradation kinetic study of capecitabine needed costly analytical techniques such as RP-HPLC. Hence, eco-friendly stability-indicating UV-visible spectrophotometry method was developed for alkaline and acidic degradation kinetic study using principle of DoE-based enhanced quality by

design approach. The potential method parameters for stability study of capecitabine was identified and assessed by risk priority number ranking and filtering as per ICH Q9 guideline. The further degradation kinetic study in alkaline and the acidic medium was carried out by DoE-based full factorial design.

MATERIALS AND METHOD

UV-Visible Spectrophotometer: Shimadzu UV-1800, UV-1700, UV- 3092, Software: UV Probe Version 2.34, two matched quartz cells with 1 cm light path, RA series REPTECH analytical balance. Temperature controller digital water bath, Janki Instruments private limited, Vadodara, Gujarat, India. Water bath sonicator, PCI Analytics Private Limited, Mumbai, India. Design Expert® software, trial version 11.0.4.0.

Reagents and Materials:

Capecitabine was procured as a gift sample from Nikshan Pharmaceuticals, Ankleshwar, Gujarat, India. Methanol was purchased from SD Fine Chemicals Limited, Mumbai. Sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Rankem laboratory, Mumbai, India. Tablet dosage forms containing 500mg of capecitabine were purchased from the local market.

PREPARATION OF SOLUTIONS:

Preparation of working standard solution of capecitabine:

An accurately weighed, 10 mg of capecitabine API powder was transferred in 10 mL volumetric flask, dissolved and diluted up to 10 mL mark with methanol. An aliquot of 1 mL was transferred in 10 mL volumetric flask and diluted up to mark with methanol to get working standard solution having the strength of 100µg/mL.

Preparation of forced degraded solutions:

The API powder of capecitabine was subjected to acid, alkaline, oxidative, dry-heat, and photolytic degradation as per guidance available in the literature. For acid hydrolysis, the capecitabine API was subjected for heating with reflux in 0.1N HCl at 40˚C for 1 hour. The alkaline hydrolysis of capecitabine API was performed in 0.1N NaOH solution at 80˚C temperature for 1 hour. For the photolytic degradation study, the capecitabine API powder was exposed to direct sunlight for 8 hours. The dry-heat degradation was carried out by heating of capecitabine API at 60˚C temperature for 3 hours.

Preliminary experimentation:

The potential method parameters were identified by conducting a brainstorming session based on preliminary experimentation and prior knowledge. The identified method parameters were

assessed for their occurrence, severity and detectability and risk priority number was calculated for each method parameters. The graph of method risk parameters versus risk priority number (RPN) was composed for the risk assessment process.

Preparation of calibration curve:

From the working standard solution of capecitabine, aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 mL were diluted to 10 mL to get standard solutions having concentration 5, 10, 15, 20 and 25µg/mL, respectively. The absorbance of each standard solution was measured at 307nm and graph of absorbance versus respective concentration was composed. The regression line equation and correlation coefficient were calculated.

Method validation:

The developed method was validated for linearity, precision, accuracy, specificity, LOD and LOQ as per ICH Q2 (R1) guideline. The linearity of the method was evaluated by repeating the procedure for calibration curve five times. The specificity study was done by measuring and comparing UV spectrum of blank, standard and sample solution. The precision study was done in terms of reproducibility, repeatability, intra and interday variations. The accuracy study was performed by calculating %recovery of the spiked standard of capecitabine in pre analyzed sample at 80, 100 and 120% levels. The limit of detection (LOD) and limit of quantitation was calculated from linearity data using equations given in ICH Q2 (R1) guideline. The robustness study was done for deliberate variation in wavelength for detection and scanning speed.

Forced degradation study:

The forced degraded sample was withdrawn, cooled, neutralized and diluted appropriately to get the measurement in calibration curve range. The sample was scanned in the range of 200-800 nm against the respective blank. The absorbances of all samples were measured at 307 nm and %degradation was calculated using the regression line equation.

Procedure for assay of tablet dosage forms:

Twenty tablets of capecitabine were weighed and powdered. The tablet powder equivalent to 100 mg of capecitabine was weighed and transferred in100mL volumetric flask. The flask was filled with 80 mL methanol and sonicated for 15 minutes for extraction of capecitabine from tablet powder. The solution was diluted up to the mark with methanol and filtered through Whatman filter paper. From the filtrate, an aliquot of 1 mL was diluted up to 10 mL with methanol. From the resulting solution, 1.5 mL was transferred in 10 mL volumetric flask and diluted up to 10 mL with methanol. The absorbance of sample solution was measure at 307nm wavelength using UV-Visible spectrophotometer and amount of capecitabine was calculated using the regression line equation.

General procedure for degradation kinetic study:

Accurately weighed 25 mg of capecitabine was transferred in 10 mL volumetric flask, dissolved and diluted up to the mark with methanol to get standard solution of capecitabine having the strength of 2500 µg/mL. The appropriate volumes solutions of 0.1N, 0.2N and 0.3N hydrochloric acid solutions were heated and equilibrated at 40˚C (30 or 50˚C) temperature using a temperature-controlled digital water bath. An aliquot of 1mL was transferred in the above solution and immediate volume adjusted to 25 mL with 0.1N HCl (or NaOH). The solution was immediately transferred in a conical flask with stopper cork. Immediately 2.5 mL solution was withdrawn, cooled, neutralized with NaOH (or HCl) in 10 mL volumetric flask and diluted up to the mark with methanol. Similarly, samples were withdrawn at the interval of 15 minutes up to 60 minutes and the same sample pre-treatment was given. The absorbance's of all samples were measured at 307nm wavelength against respective blank and concentration of remained capecitabine was calculated using the regression line equation. The order of reaction, rate constant, half-life, shelf-life and % degradation of capecitabine was calculated.

DoE-based full factorial design for hydrolytic degradation kinetic study in acidic medium:

The 3^2 full factorial design was applied for acidic degradation kinetic study of capecitabine at 30, 40 and 50˚C temperature in different strength of HCl. Total thirteen experimental runs including four centre points were performed laboratory using procedure of degradation kinetic study (section 2.9) and rate constant and %degradation was calculated for each run in design expert software. The data was entered against the respective experimental run and analyzed by ANOVA and contour response surface plot.

DoE-based full factorial design for hydrolytic degradation in alkaline medium:

Again DoE base full factorial design was applied for degradation kinetic study of capecitabine in different strength of sodium hydroxide at 30, 40 and 50˚C temperature. Thirteen experimental runs suggested by design expert software were performed in the laboratory using the procedure of degradation kinetic study (section 2.9). The %degradation and rate constant of each trial was measured and entered in software against the respective run. The kinetic data were analyzed by statistical tools such as ANOVA and contour response surface plot.

RESULTS AND DISCUSSION:

According to upcoming ICH Q14 guideline, the development of an analytical method should be more emphasised on method behaviour understanding and control to get desired output or results. The analytical method behaviour understanding and control can be easily achieved by the implementation of enhanced quality by design approach based on principles of design of

experiments and quality risk management as per ICH guideline Q8, Q9 and Q10. In the literature, stability-indicating RP-HPLC method was only reported for degradation kinetic study of capecitabine. But the RP-HPLC method is a relatively costly and time-consuming method for the stability study of the drug. Hence, economical and eco-friendly stability-indicating UV-Visible spectrophotometry was developed for degradation kinetic study of capecitabine using enhanced quality by design approach based on the principle of design of experiments. The implementation quality by design approach was started with defining of analytical target profile and critical method attributes.

Analytical Target Profile & Critical Method Attributes:

The development of stability-indicating UV-visible spectrophotometry for estimation of capecitabine free from the interference of degradation products formed indifferent stress condition. For the development of the target method, % degradation of capecitabine and rate constant for degradation was selected as critical method attributes. The % degradation of capecitabine more than 5% would be considered as significant change as per the ICH Q1A guideline for stability study of the drug substance.

Preliminary Trials for Identification of Potential Method Parameters:

The capecitabine API was subjected to degradation in 0.1N HCl, 0.1N NaOH, 1%v/v H_2O_2, dry-heat in hot-air oven and photolysis by sunlight exposure. The hydrolytic degradation was conducted at different temperature (30, 40, & 50˚C) for checking of temperature effect on degradation of capecitabine and % degradation of capecitabine was increased as temperature increased by 10˚C. The sample of the degraded sample was scanned in UV-Visible spectrophotometer and %degradation of capecitabine was measured. The degradation of capecitabine was found to be significant in acidic, alkaline and oxidative condition. The degradation of capecitabine was found to be faster in hydrolytic stress condition as compared to oxidative condition. Hence, the hydrolytic degradation pathway was selected for degradation kinetic study of capecitabine for estimation of %degradation and rate constant. The spectrum of capecitabine showed maximum absorbance at 210, 240 and 307 nm respectively but in the spectrum of alkaline degraded sample wavelength maxima was found to be 307 nm. Hence, the wavelength of 307 nm was selected. The instrumental variation and analyst variations were also checked during experimentation for method reproducibility. The sampling was also done in different volume glassware and volumes to check variation in results of the stability study. The degraded sample was also scanned at different scanning speed for checking of instrumental variation on results of the method.

Identification and Assessment of Potential Method Parameters:

The potential method parameters for the development of stability-indicating assay method was identified by conducting a brainstorming session based on preliminary experiments and prior literature. The identified method parameters were categorized and listed in the cause-effect diagram (see figure I). The risk assessment of identified method parameters was done by allotting risk priority number according to their occurrence, severity and detectability during preliminary experimentations according to the guidance given in ICH Q9 guideline for quality risk management. The graph of potential method parameters versus risk priority (RPN) score was composed for risk assessment (see figure III). The potential method parameter has a risk priority number more than 60 was considered as critical method parameter for the development of the target method. The risk priority number of hydrolytic degradation and temperature were found 200 that indicated these were a high-risk factor (red colour in the graph) need to be studied for the development method. The RPN score of the wavelength of detection and oxidative degradation was found to be more than 96 and 72 respectively that implied these risk factors were moderately severe (yellow colour in the graph) for development of target method and need to be fixed. The other method parameters such as sampling variation, analyst variation, instrument variation, scanning speed etc. (green colour in the graph) were found to be insignificant for development of the method. The spectrum of acidic, alkaline and oxidative degradation was shown in figure IV.

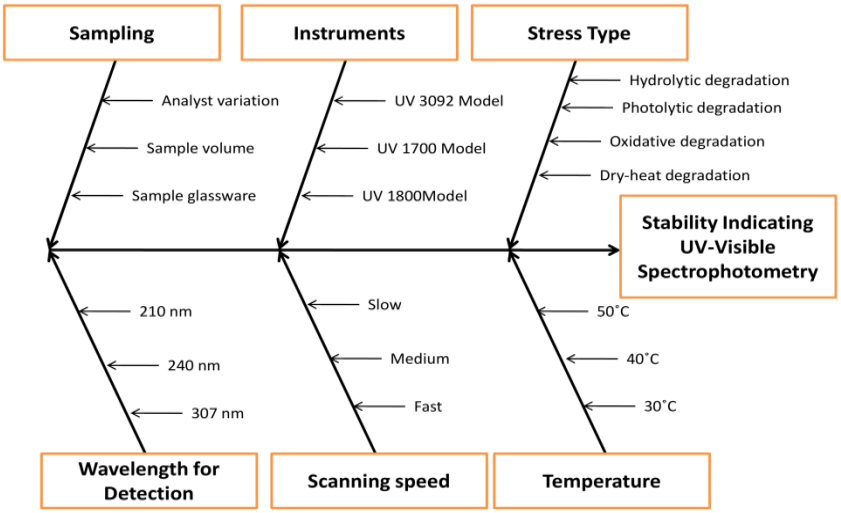

Figure II: The cause and effect diagram showing a list of potential method parameters for the development of stability-indicating assay method

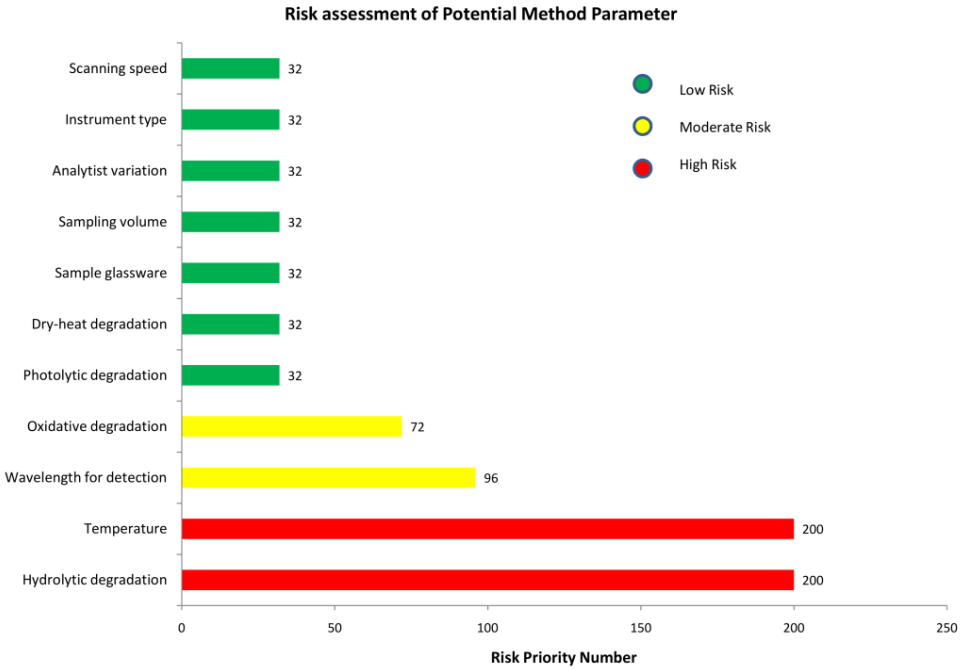

Figure III: Graph of potential risk parameters versus risk priority number for quality risk assessment of potential method parameters for the development of the method

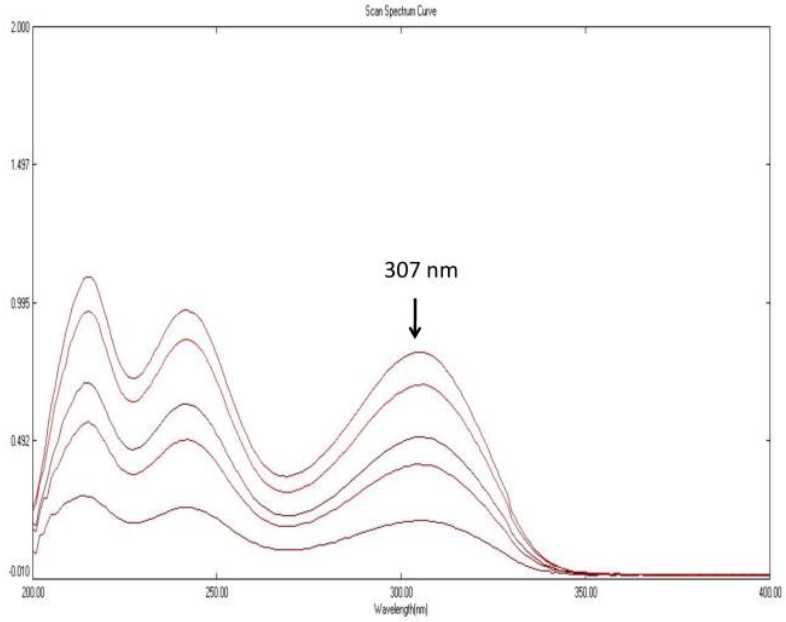

Figure IV: Overlaid UV-spectrum for calibration curve of capecitabine over the concentration range of 5-25 µg/mL.

Calibration Curve of Capecitabine:

The capecitabine API was showed a linear relationship with absorbance in the range of 5-25µg/mL with a correlation coefficient of 0.9970. The data of linearity was depicted in table I and the calibration curve was shown in figure IV.

Table I: Results of linearity for CPC

Parameters	Results
Linearity range	5-25µg/ml
Regression line equation	y=0.034x+0.038
Slope ± S.D.(n=5)	0.034 ± 0.00506
Y –intercept ± S.D.(n=5)	0.0388 ± 0.0099
Correlation coefficient (R^2)	0.997

Method Validation:

The method was validated for specificity study and there was no additional peak observed in sample and standard. The overlain UV spectra of standard and sample were found to be highly correlated. The method was showed linearity in the range of 5-25µg/mL with a correlation coefficient of 0.9970 for capecitabine. The data of linearity is showed in Table I. The % RSD for precision studies such as reproducibility, repeatability, intra-day and inter-day variation was found to less than 2.0 that indicated method is precise. The %recovery of capecitabine was found to be in the range of 98-102% that implies the method is accurate. The %RSD of all robustness study was found to be less than 2 that indicated method is robust. The LOD and LOQ for capecitabine were found to be 0.79 µg/mL and 2.39 µg/mL respectively. The summary of method validation parameters is shown in table II.

Table II: Summary of validation parameters

Sr. No.	Validation parameters	Results
1.0	Linearity range	5-25 µg/mL
2.0	Correlation co-efficient	0.9970
3.0	Precision (%CV)	
3.1	Repeatability of sample measurement (n=7)	0.85
3.2	Repeatability of sample preparation (n=7)	0.98
3.3	Intra-day precision (n=3)	1.04 – 1.44
3.4	Inter-day precision (n=3)	1.12 – 1.55
4.0	%Recovery (n=3)	99.89 - 101.32
5.0	Limit of Detection (LOD)	0.79 µg/mL
6.0	Limit of Quantitation (LOQ)	2.39 µg/mL
7.0	Specificity	Specific

Forced Degradation Study of Capecitabine:

The forced degraded sample of capecitabine was analyzed by the developed method and degradation was found to be faster in hydrolytic conditions. The % degradation of capecitabine was found to be 38.96 and 20.96% respectively in acidic and alkaline hydrolytic condition. In the oxidative degradation study, the %degradation of capecitabine was found to be 11.52%. The degradation of capecitabine in dry-heat and photolytic conditions was found to be less than 5% that indicated no significant change in capecitabine as per ICH Q1A guideline. The data of forced

degradation study is shown in table III. The UV spectrum showing degradation in 0.1 N HCl, 0.1 N NaOH and 1% H_2O_2 is shown in figure V.

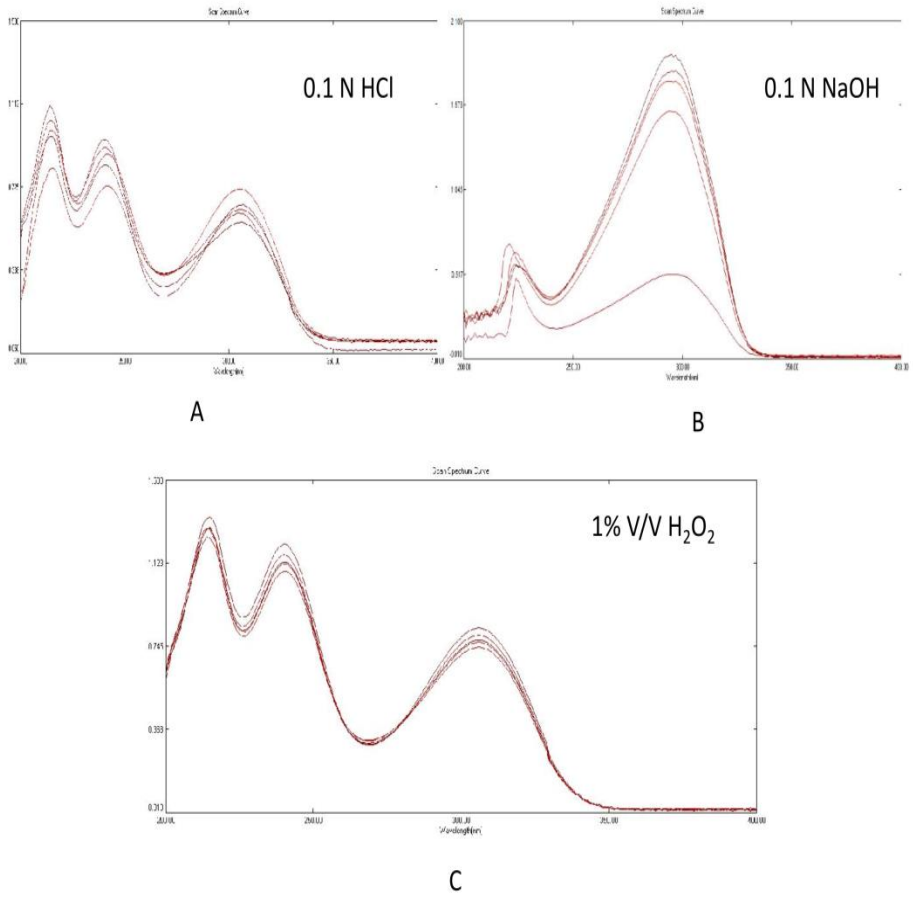

Figure V: Overlaid UV spectra of capecitabine showing forced degradation of capecitabine in (a) 0.1 N HCl at 40˚C (b) 0.1 N NaOH at 40˚C temperature (c) 1.0 %V/V hydrogen peroxide at 40˚C

Table III: Results of forced degradation study

Forced degradation	Stress Condition	Initial conc. of CPC (µg/mL)	Final conc. of CPC (µg/mL)	% CPC remain	% Degradation
Acidic hydrolysis (0.1N HCl)	1.0 hrs heating, 40˚±2˚C	25	15.26	61.04	38.96
Alkaline hydrolysis (0.1N NaOH)	1.0 hrs heating, 80˚±2˚C	25	19.76	79.04	20.96
1.0 % H_2O_2	1.0 min at 27˚±2˚C	25	22.12	88.48	11.52
Photolytic degradation-	direct sunlight exposure for 8 hours	25	24.20	96.8	3.2
Dry-heat degradation	60˚C for 3 hours	25	24.60	98.4	1.6

Assay of Tablet Dosage Forms of Capecitabine:

The %assay values of three different brand of capecitabine tablet dosage forms were found to be in the range of 95-105% range which was following pharmacopoeial standards for assay of tablet dosage forms. The results for the assay of capecitabine is shown in table IV.

Table IV: Results for assay of different tablet dosage forms of capecitabine

Formulation		CPC
Brand A	Amount labeled (mg)	500
	Amount found (mg)	515
	% Amount found ± SD (n=3)	103.0 ± 0.85
Brand B	Amount labeled (mg)	500
	Amount found (mg)	493.75
	% Amount found ± SD (n=3)	98.75 ± 1.02
Brand C	Amount labeled (mg)	500
	Amount found (mg)	513.75
	% Amount found ± SD (n=3)	102.75 ± 0.82

DoE-based Full-factorial Design for Acid-hydrolytic Degradation Kinetic Study:

In the risk assessment study, the degradation of capecitabine was found to be significant in hydrolytic stress condition. Hence, degradation kinetic study was carried out in acidic and alkaline medium at three different temperature. The degradation kinetic study of capecitabine was carried out in 01, 0.2 and 0.3 N HCl at three different temperatures 30, 40 and 50ºC using full factorial design. The suggested thirteen experimental trials including four centre points were performed in the laboratory (see Table V) and % degradation and rate constants were measured for each trial. The data of degradation kinetic study was entered in design expert software against respective runs and analyzed by ANOVA. According to the data of ANOVA table VI, the temperature, strength of HCl and their interaction were found to be significant factors for the degradation of capecitabine. The two-factor interaction model was found to be significant for the acidic degradation study. The 2D and 3D contour plots for rate constant and % degradation of capecitabine in acidic medium is shown in figure VI and VII respectively.

Table V: Design metrics for hydrolytic degradation in acidic medium

Std	Run	Factor 1 A:Temperature	Factor 2 B:Strenght of Hydrochloric acid	Response 1 Rate constant	Response 2 %degradation
		˚C	N	minute-1	%
4	1	30	0.2	0.329	26.4
7	3	30	0.3	0.4614	23.8
1	7	30	0.1	0.2333	7.22
2	2	40	0.1	1.0156	43.8
13	4	40	0.2	1.1926	53.32
8	6	40	0.3	1.3881	64.84
12	8	40	0.2	1.1742	52.14

10	9	40	0.2	1.1754	53.74
5	10	40	0.2	1.1824	52.98
11	12	40	0.2	1.1845	53.14
3	5	50	0.1	1.2512	47.6
6	11	50	0.2	1.8327	65.8
9	13	50	0.3	2.5154	80.6

Table VI: ANOVA data for rate constant and % degradation in acidic medium

Analysis of variance table [Partial sum of squares - Type III]: Rate Constant						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob. > F**	
Model	4.36	5	0.87	129.24	< 0.0001	Significant
A-Temperature	3.49	1	3.49	516.92	< 0.0001	
B-Strength of Hydrochloric acid	0.58	1	0.58	85.86	< 0.0001	
AB	0.27	1	0.27	39.76	0.0004	
A^2	0.024	1	0.024	3.62	0.0990	
B^2	2.014E-003	1	2.014E-003	0.30	0.6019	
Residual	0.047	7	6.750E-003			
Analysis of variance table [Partial sum of squares - Type III]: % Degradation						
Model	4452.11	5	890.42	84.74	< 0.0001	significant
A-Temperature	3109.02	1	3109.02	295.89	< 0.0001	
B-Strength of Hydrochloric acid	831.20	1	831.20	79.11	< 0.0001	
AB	67.40	1	67.40	6.41	0.0391	
A^2	310.87	1	310.87	29.59	0.0010	
B^2	15.77	1	15.77	1.50	0.2602	
Residual	73.55	7	10.51			

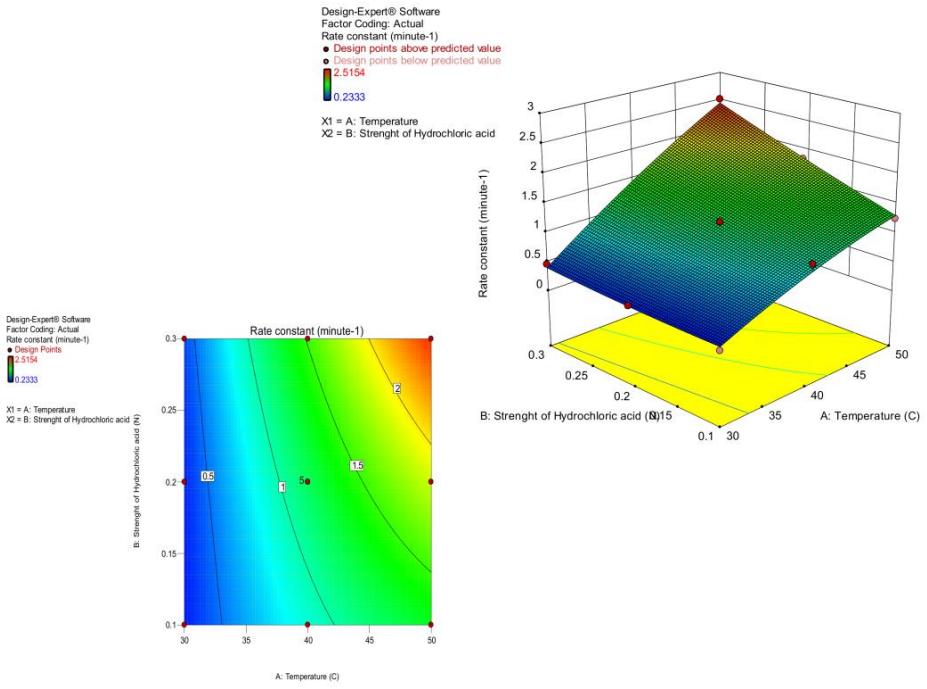

Figure VI: 2D and 3D contour plot showing the interaction of temperature and strength of HCl for the rate constant for acidic degradation kinetic study of capecitabine

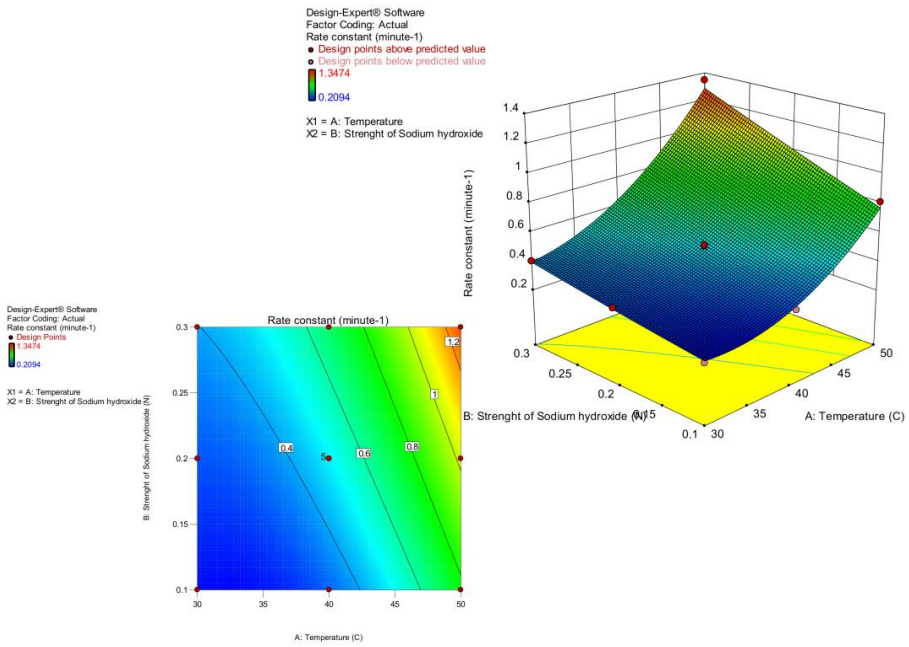

Figure VIII: D and 3D contour plot showing the interaction of temperature and strength of HCl for the rate constant for alkaline degradation kinetic study of capecitabine

Doe-Based Full-Factorial Design for Alkaline-Hydrolytic Degradation Kinetic Study:

The alkaline degradation kinetic study was carried out in 0.1, 0.2 and 0.3 N NaOH at three different temperatures using full factorial design. The thirteen experimental runs were performed in the laboratory suggested by the design expert software and % degradation and rate constant for each run was measured (see Table VII). The data were analyzed by ANOVA and results showed the main effects of temperature and strength of NaOH, two-way interaction of temperature and strength of NaOH and quadratic effect of temperature was found to be significant for the degradation kinetic study of capecitabine in alkaline condition (see Table VIII). The 2D and 3D contour plots for % degradation and rate constants are shown in figure VIII and IX respectively.

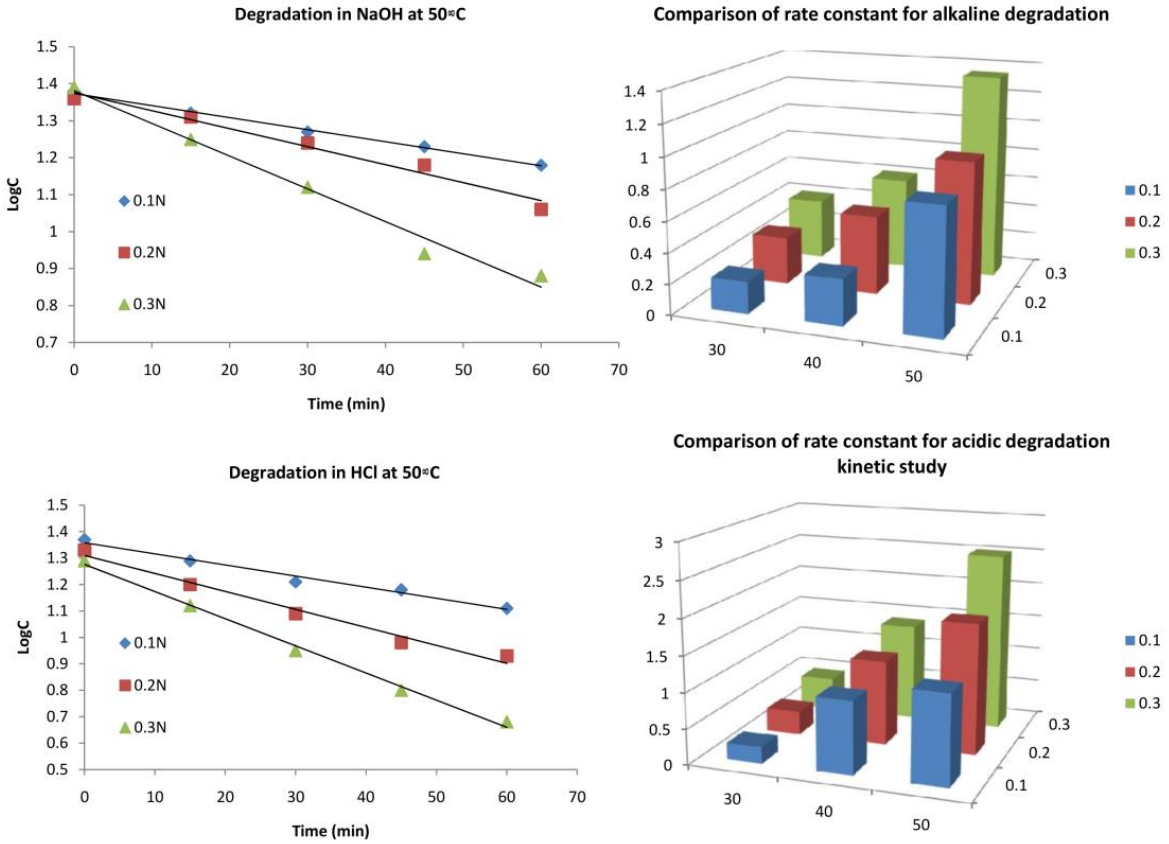

Figure X: Comparison of acidic and alkaline hydrolysis of capecitabine for % degradation and degradation rate constant

Table VII: Data metrics for hydrolytic degradation in alkaline medium by FFD

Std	Run	Factor 1 A:Temperature	Factor 2 B:Strenght of Sodium hydroxide	Response 1 Rate constant	Response 2 %degradation
		˚C	N	minute-1	%
1	7	30	0.1	0.2094	14.52
4	1	30	0.2	0.3118	19.04
7	3	30	0.3	0.4034	25.15
2	2	40	0.1	0.2993	20.88
13	4	40	0.2	0.5141	24.36
12	8	40	0.2	0.5098	24.12
10	9	40	0.2	0.5147	24.78
5	10	40	0.2	0.5074	24.17
11	12	40	0.2	0.5094	24.24
8	6	40	0.3	0.6054	30.15
3	5	50	0.1	0.8125	39.48
6	11	50	0.2	0.924	54.08
9	13	50	0.3	1.3474	69.68

Table VIII: ANOVA data for rate constant and % degradation in alkaline medium

Analysis of variance table [Partial sum of squares - Type III]: Rate Constant						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob. > F**	
Model	1.08	5	0.22	68.14	< 0.0001	Significant
A-Temperature	0.78	1	0.78	244.62	< 0.0001	
B-Strength of Sodium hydroxide	0.18	1	0.18	56.20	0.0001	
AB	0.029	1	0.029	9.15	0.0193	
A^2	0.081	1	0.081	25.57	0.0015	
B^2	9.818E-005	1	9.818E-005	0.031	0.8654	
Residual	0.022	7	3.177E-003			
Analysis of variance table [Partial sum of squares - Type III]: % Degradation						
Model	2827.92	5	565.58	94.47	< 0.0001	significant
A-Temperature	1821.09	1	1821.09	304.17	< 0.0001	
B-Strength of Sodium hydroxide	418.33	1	418.33	69.87	< 0.0001	
AB	95.75	1	95.75	15.99	0.0052	
A^2	395.63	1	395.63	66.08	< 0.0001	
B^2	2.36	1	2.36	0.39	0.5504	
Residual	41.91	7	5.99			

Energy of Activation for Hydrolytic Degradation:

The hydrolytic degradation kinetic was found to be first order by graphical methods. The % degradation of capecitabine was found to be increased as temperature and the strength of acid or base increased (see figure X). The % degradation, rate constant, half-lives and shelf-lives were calculated for each point and summarized in Table IX. The energy of activation for hydrolytic degradation study was calculated using Arrhenius equation ($k = Ae^{-Ec/RT}$) and the energy of activation was found to be deceased as temperature and strength of acid/base increased.

3.8 Prediction of Degradation at Different Temperature:

The prediction of % degradation, rate constants and half-lives of capecitabine in hydrolytic condition were done using 2D response obtained by design expert software after degradation kinetic data analysis. The predicted values were confirmed by experimentations and all results were found in good agreement with the predicted values. The values of predicted results are depicted in table X.

Table IX: Summary of alkaline and acidic hydrolytic degradation kinetic study data of CPC

Temp.(°C)	**Strength of Acid/Base**	**Average degradation rate constant $K \times 10^{-2}(min^{-1})$**	**Half-life(min.)**	**Self-life (min.)**	**Order of reaction**
Alkaline degradation study in NaOH					
30 ± 2	0.1	0.2094	330.82	50.12	First
	0.2	0.3118	222.25	33.67	

	0.3	0.4034	171.78	26.02	
40 ± 2	0.1	0.2993	231.48	35.07	First
	0.2	0.5141	134.79	20.34	
	0.3	0.6054	114.49	17.34	
50 ± 2	0.1	0.8125	85.28	12.92	First
	0.2	0.9240	74.99	11.36	
	0.3	1.3474	51.43	7.79	
Acidic degradation study in HCl					
30 ± 2	0.1	0.2333	297.04	45.06	First
	0.2	0.3290	210.62	31.91	
	0.3	0.4614	150.18	22.75	
40 ± 2	0.1	1.0156	68.21	10.33	First
	0.2	1.1926	58.10	8.80	
	0.3	1.3881	49.92	7.56	
50 ± 2	0.1	1.2512	55.38	8.39	First
	0.2	1.8327	37.81	5.72	
	0.3	2.5154	27.54	4.17	

Table X: Prediction of hydrolytic degradation in HCl at different temperature

Temperature (°C)	**Strength of HCl/NaOH (N)**	**Rate constant (min^{-1}) $K \times 10^{-2}$**	**Half –life (minute)**	**% Degradation**
Acidic degradation study				
	0.1	0.4585	151.14	19.64
32	0.2	0.5440	127.38	30.56
	0.3	0.6669	103.91	36.22
	0.1	0.7592	91.28	34.56
37	0.2	0.9748	71.09	47.44
	0.3	1.2261	56.52	55.23
	0.1	1.0169	68.14	44.04
42	0.2	1.3487	51.38	58.74
	0.3	1.7931	38.64	70.13
Alkaline degradation study				
	0.1	0.2189	316.58	15.10
32	0.2	0.3151	219.93	18.10
	0.3	0.4303	161.05	23.15
	0.1	0.2652	216.31	14.50
37	0.2	0.4071	170.22	20.55
	0.3	0.5664	122.35	28.62
	0.1	0.4050	171.11	20.80
42	0.2	0.5799	119.5	28.81
	0.3	0.8079	85.77	40.79

CONCLUSION

The stability-indicating UV-Visible spectrophotometry was developed and validated for degradation kinetic study of capecitabine. The degradation kinetic and forced degradation results of present methods were found in good agreement with reported RP-HPLC methods. Hence, the developed method can be used as an economical and eco-friendly tool for stability study of

capecitabine without the interference of degradation products formed in different stress conditions. The developed method was found to be precise, robust, accurate, sensitive, specific and reproducible for the stability study of capecitabine as per ICH Q2 (R1) guideline. The developed method was developed using enhanced quality by design based on principles of quality risk management and design of experiments that implies the developed method is in regulatory compliance with ICH Q8, Q9, Q10 and Q14 guidelines. The developed method can be used for routine stability study and quality control of capecitabine in small and large scale pharmaceutical

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