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DEVELOPMENT AND VALIDATION OF NEW UV SPECTROSCOPIC METHODS FOR THE ESTIMATION OF CAPECITABINE IN BULK AND FORMULATIONS

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ABSTRACT

Capecitabine is a fluoropyrimidine carbamate, designed as 'pro-drug' to the cytotoxic agent 5-fluorouracil (5-FU) meant to be administered orally. Capecitabine is used as first line monotherapy for metastatic colorectal cancer. In the present study simple, rapid, accurate UV spectrophotometric methods were developed and validated for the estimation of capecitabine in bulk and its formulations as per ICH guidelines. Three solvent systems viz., 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) were tried. The results suggest that the developed methods shows linearity over the concentration range of 2-24µg/ml with a correlation coefficient of 0.9999. All the developed methods were statistically validated for accuracy, precision, linearity, robustness, and ruggedness as per ICH guidelines. The % RSD values for validated methods were found to be less than 1.5 and methods will find application in routine analysis of drug formulations containing capecitabine.

KEYWORDS: Capecitabine, UV-spectrophotometric, 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3).

INTRODUCTION

Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers[1] and is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR), which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue.[2,3] 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. Chemically it is 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] – cytidine (figure 1) with empirical formula $C_{15}H_{22}FN_3O_6$ and the molecular weight of 359.35 g/mol.[4, 5]

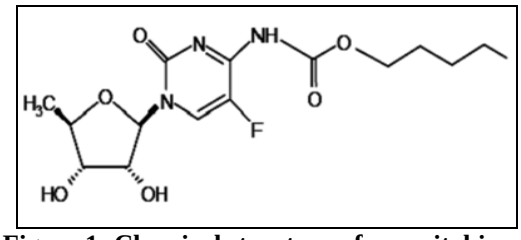

Figure 1: Chemical structure of capecitabine.

A very few methods appeared in the literature for the assay of capecitabine in biological fluids and pharmaceutical formulations viz., HPLC[6-9], LC-UV[10, 11], LC-MS[12], and LMS/MS[10,13] methods. Hence in the present work it was aimed to develop and validate accurate, precise, simple and rapid UV spectroscopic methods for the estimation of capecitabine in bulk and its formulations as per ICH guidelines.

MATERIALS AND METHODS

MATERIALS

Capecitabine gift sample was obtained from Shilpa antibiotic Pvt Ltd, Raichur. Methanol, Hydrochloric acid and Sodium hydroxide were procured from S.D Fine chemicals Mumbai, double distilled water was used throughout the experiments. All the other chemicals used were of analytical grade.

METHODS

Determination of absorption maxima (λ max)

Preparation of capecitabine standard stock solution (1000µg/ml): Weighed accurately about 50 mg of capecitabine working standard and transferred to a 50 ml volumetric flask. Add 40 ml of 0.1N NaOH and shake for 5 minutes to dissolve and dilute to volume with 0.1N NaOH. Similarly prepare standard stock solutions in 0.1N HCl and Methanol: Water (1:3) solvent systems.

Preparation of capecitabine sample solution: Transferred aliquots of standard stock solution into a series of 10 ml volumetric flask and dilute with 0.1N NaOH to get desired concentrations. Similarly sample solutions were prepared in 0.1N HCl and Methanol: Water (1:3) solvent systems.

Procedure: The sample solutions were subjected for UV scanning in the range of 200-380 nm using double beam UV Spectrophotometer and determine the absorption maxima of capecitabine. Similarly determine the absorption maxima of capecitabine in 0.1N HCl and Methanol: Water (1:3) solvent systems**.**

Determination of linearity range

Procedure: Standard solutions of capecitabine in the concentration range of 4-40 µg / ml were prepared in 0.1N NaOH and absorbance was measured at 292.8nm taking the 0.1N NaOH as the blank. Similarly absorbances of capecitabine in the concentration range of 4-40 µg / ml in 0.1N HCl, and Methanol: Water (1:3) were measured at 304nm and 300.8nm respectively using 0.1N HCl, and Methanol: Water (1:3) solvent systems as blank.

Preparation of calibration curve

Procedure: Appropriate aliquots from standard capecitabine stock solutions were transferred to series of 10 ml volumetric flasks. The volume was adjusted to the mark with 0.1N NaOH to obtain concentrations of 4, 8, 12, 16, 20 and 24µg/ml and measure the absorbance at 292.8nm. Similarly a set of same concentrations were prepared in 0.1N HCl and Methanol: Water (1:3) measure the absorbance at 304nm and 300.8nm respectively against respective solvent systems as blank. The concentration vs absorbance values were plotted and interpreted statistically.

Validation

Accuracy

The accuracy was evaluated applying the proposed methods to the analysis formulations with known amounts of drug. The accuracy was calculated as the percentage of the drug recovered from the formulations.

Preparation of standard capecitabine solution (for bulk): Transfer accurately weighed about 50 mg of capecitabine working standard to a 50 ml volumetric flask, add about 40 ml of 0.1N NaOH to dissolve, dilute up to the mark with 0.1N NaOH and mix. Aliquots of stock solution were further diluted with 0.1N NaOH to get desired concentrations. Similarly standard solutions were prepared in other solvent systems viz., 0.1N HCl, Methanol: Water (1:3).

Preparation of capecitabine sample preparation (for tablets): Weigh accurately 5 tablets and grind in a mortar and transfer equivalent to 50 mg of capecitabine into a 50 ml volumetric flask, add 40 ml of 0.1N NaOH and shake it for 1h. Dilute to volume with 0.1N NaOH mix the contents and filter through 0.45 µm membrane filter. Transfer aliquots of the filtrate to 25ml volumetric flask and dilute to volume with the 0.1N NaOH to get desired concentration. Similarly sample solutions were prepared in other solvent systems viz., 0.1N HCl, Methanol: Water (1:3).

Procedure: Recovery studies were carried out by adding known amount of standard drug (40% and 20%) to the sample solution measure the absorbance and calculate the amount from the calibration curve. The % recovery was calculated in terms of % RSD and it should be less than 2%.

Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter day). Repeatability was evaluated assaying 3 determinations at the same concentration (10µg/ml), during the same day, under the same experimental conditions. Intermediate precision was analyzed comparing the assays in 3 determinations at the same concentration (10µg/ml) during 3 different days. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD).

Sample preparation (for tablets): Weigh accurately 5 tablets and grind in a mortar and transfer equivalent to 50 mg of capecitabine into a 50 ml volumetric flask, add 40 ml of 0.1N NaOH and shake it for 1h. Dilute to volume with 0.1N NaOH mix the contents and filter through 0.45 µm membrane filter. Transfer aliquots of the filtrate to 25ml volumetric flask and dilute to volume with the 0.1N NaOH to get desired concentration. Similarly sample preparations were prepared in other solvent systems viz., 0.1N HCl, Methanol: Water (1:3).

Procedure: Intraday precision was determined by analyzing capecitabine for three times in the same day (morning, afternoon, evening) at respective absorption maxima using respective solvent systems. Interday precision was determined by analyzing daily once (morning) for three days at respective absorption maxima using respective solvent systems. The % RSD values were calculated and it should be less than 2%.

LOD and LOQ

LOD/LOQ parameters are not a requirement for drug assay, however it is always useful to demonstrate that the analyses are being conducted in a region which is above the LOQ value. The LOD and LOQ were calculated based on the standard deviation of the response (y-intercepts of regression lines) and the slope using three independent analytical curves, as denied by ICH.

$$\textbf{LOD (µg/ml)} = 3.3 \times \frac{\sigma}{s} \quad \textbf{LOQ (µg/ml)} = 10 \times \frac{\sigma}{s}$$

Where σ - Standard deviation of the response; s – Slope ratio curve.

Procedure: The lowest possible concentration where the drug capecitabine show response was determined in all the solvent systems. The absorbance at this concentration was measured in triplicate in respective solvent systems at respective absorption maxima. The LOD/LOQ was calculated by using formulae from the data obtained.

Robustness and Ruggedness

Robustness

Robustness of the proposed methods were determined by the analysis of samples and standard solutions (10 µg/ml) at different wavelengths (±5nm), at different solution temperatures (refrigeration condition 2-8 ºC and 37ºC). To assess the stability of drug, the stability study was performed maintaining the drug working solution in respective solvent systems for 48h protected from light, looking for the decrease of absorbance compared with those of freshly prepared solutions.

Procedure: Appropriate concentrations of capecitabine from bulk and formulations were prepared in respective solvent systems. Analysis was carried out at three different wavelengths (actual and ± 5 nm). Amount found was calculated at three different wavelengths in terms of % RSD and values should be less than 2%.

Ruggedness

Ruggedness is not addressed in the ICH documents. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from analyst to analyst and instrument to instrument.

Procedure: Appropriate concentrations of capecitabine from bulk and formulations were prepared in respective solvent systems. Analysis was carried out by two different analysts and also two instruments. Amount found was calculated at three different wavelengths in terms of % RSD and values should be less than 2%.

RESULTS AND DISCUSSION

Simple, rapid, economic, accurate, precise and sensitive UV spectrophotometric methods were developed and validated as per ICH guideline and USP 2000 for the estimation of capecitabine in bulk and formulatons. Three different solvent systems viz., 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) was selected. The developed methods were further validated for accuracy, precision, LOD, LOQ, specificity, robustness and ruggedness with statistical data.

The absorption maxima (λmax) with characteristic peak for capecitabine were found at 292.8nm, 304.0nm and 300.8nm for 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) respectively. These absotption maxima were used to detemine the linearity and it was shown linear relationship with correlation coefficient of 0.9999; 0.9999 and 0.9999 for 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) respectively in the concentration range of 2-24 µg/ml. The spectra and datas were shown in figure 2, 3 and table1.

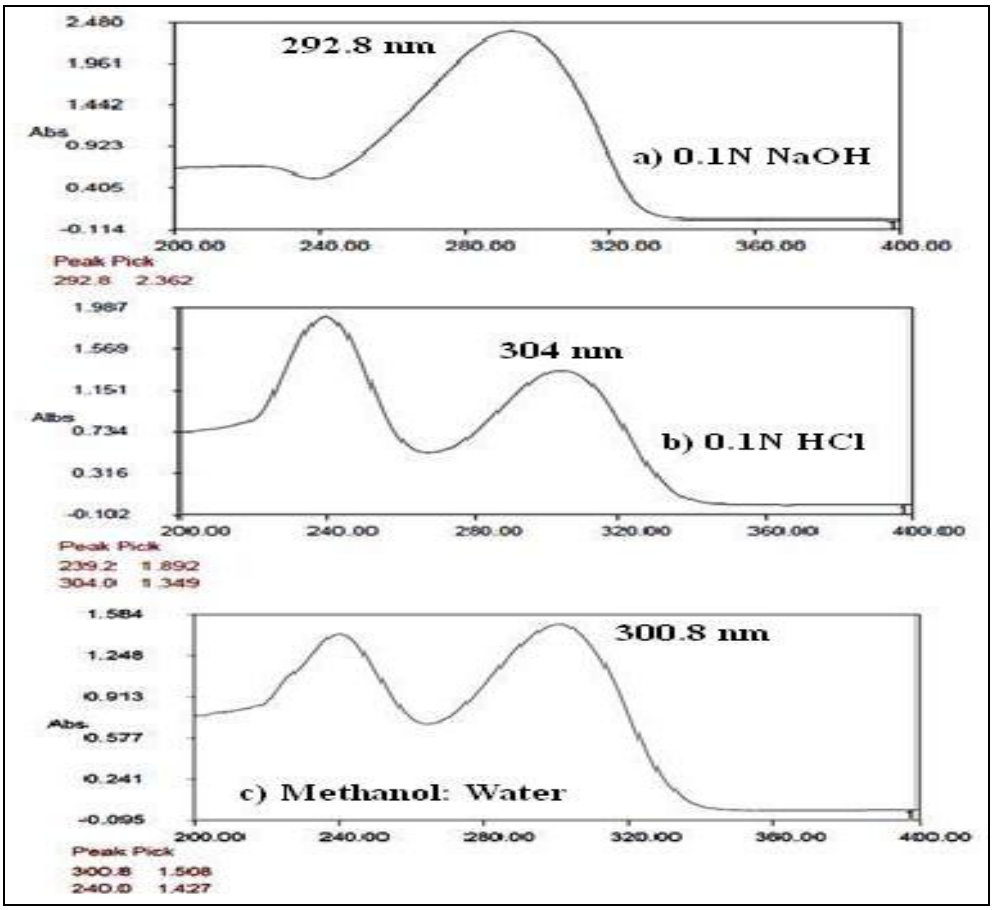

Figure 2: Absorption maxima of capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems.

Table 1: Linearity range curve of capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems.

Linearity range curve of capecitabine			
Conc. (µg/ml)	**0.1N NaOH Absorbance*** ± SD	**0.1N HCl Absorbance*** ± SD	**Methanol: Water (1:3) Absorbance*** ± SD
4	0.0672 ±0.0004	0.0677±0.0003	0.0670 ± 0.003
8	0.118± 0.0021	0.123± 0.0021	0.124± 0.0022
12	0.178 ± 0.0016	0.183 ± 0.0016	0.188 ± 0.0016
16	0.237 ± 0.0010	0.242 ± 0.0010	0.243 ± 0.0011
20	0.302 ± 0.0049	0.307 ± 0.0049	0.308 ± 0.0048
24	0.362 ± 0.0020	0.368 ± 0.0020	0.369 ± 0.0021
28	0.382 ± 0.0011	0.421 ± 0.0011	0.387 ± 0.0011
32	0.412 ± 0.0032	0.441 ± 0.0032	0.412 ± 0.0032
36	0.472 ± 0.0023	0.473 ± 0.0023	0.462 ± 0.0023
40	0.512 ± 0.0033	0.511 ± 0.0033	0.489 ± 0.0023

***Average of six determinations.**

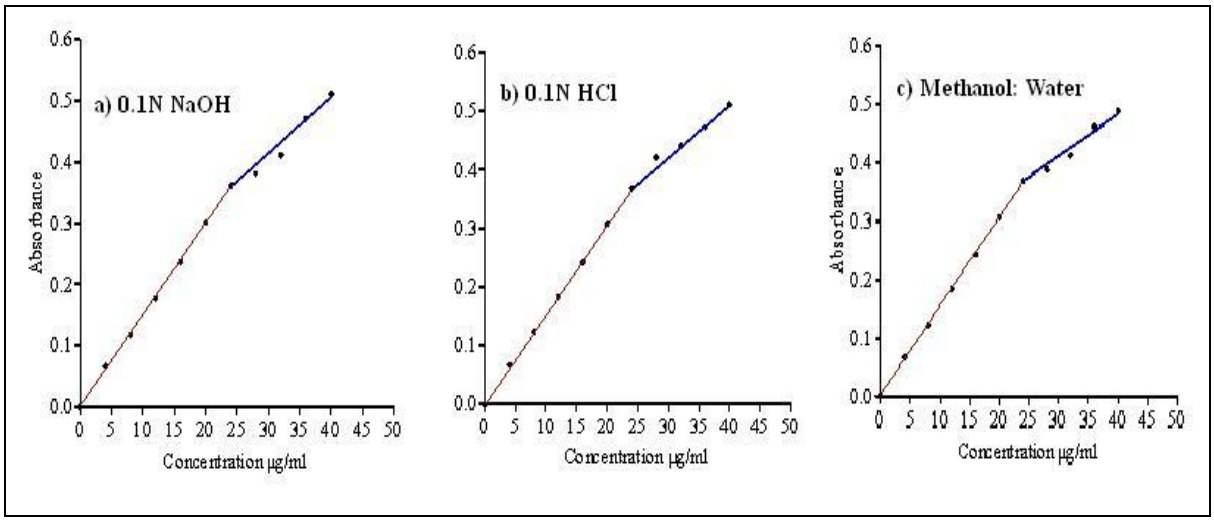

Figure 3: Linearity range of capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems in observed absorption maxima.

The calibration curve for capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems were prepared in the concentration range of 4-24µg/ml. In all the solvent systems the P value is < 0.0001 indicate proposed methods were found to be statistically significant. The calibration curve data and statistical data were shown in table 2, 3 and calibration curve in figure 4.

Table 2: Calibration curve data of capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems.

Conc. (µg/ml)	**0.1 N NaOH Absorbance*** ± SD	**0.1 N HCl Absorbance*** ± SD	**Methanol: Water (1:3) Absorbance*** ± SD
0	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
4	0.0622±0.0012	0.0658±0.0015	0.0637±0.0018
8	0.1180±0.0015	0.1240±0.0019	0.1230±0.0026
12	0.1780±0.0019	0.1840±0.0021	0.1830±0.0029
16	0.2370±0.0021	0.2470±0.0024	0.2460±0.0032
20	0.2970±0.0026	0.3080±0.0026	0.3070±0.0023
24	0.3560±0.0018	0.3690±0.0021	0.3680±0.0019

***Average of six determinations.**

Table 3: Statistical data of calibration curve for capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems.

Parameters	0.1 N NaOH	0.1 N HCl	Methanol: Water (1:3)
λ_{max} (nm)	292.8	304.0	300.8
Beer's range (µg/ml)	2-24 µg/ml	2-24 µg/ml	2-24 µg/ml
Molar absorptivity ($mol^{-1}cm^{1}$)	1.714×10^{4}	1.716×10^{4}	1.711×10^{4}
Best fit valves			
Slope	0.0147 ± 0.000057	0.0153 ± 0.000050	0.01531 ± 0.000076
Y-intercept when X=0.0	0.00082 ± 0.00082	0.00078 ± 0.00072	0.001714 ± 0.0011
X-intercept when Y=0.0	-0.05554	-0.05135	-0.1120
1/Slope	67.61	65.36	65.33
95% CI			
Slope	0.01464 to 0.01494	0.01517 to 0.01543	0.01511 to 0.01550
r^2	0.9999	0.9999	0.9999
P valve	< 0.0001	< 0.0001	< 0.0001

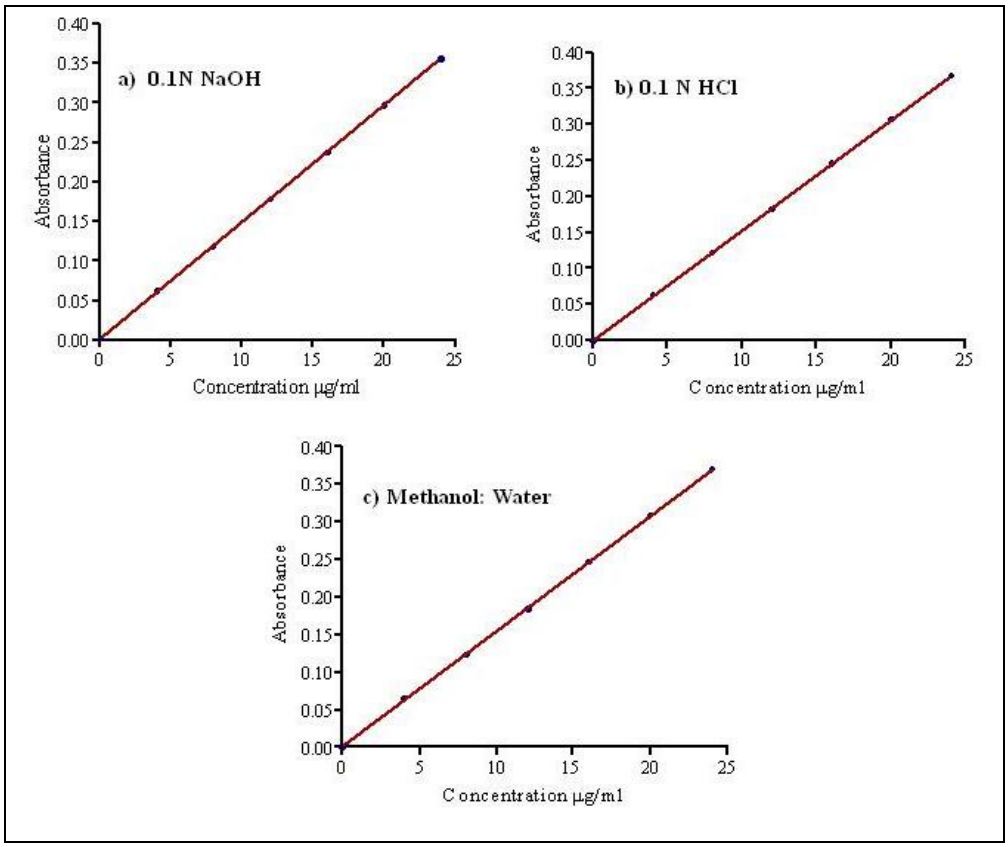

Figure 4: Calibration curve of capecitabine in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems.

The accuracy was found to be in the range of 98.7-100.5; 99.2%-100.5%; 99.4%-100.6% in 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems respectively for the estimation of capecitabine in bulk and the data were given table 4.

Table 4: Data showing accuracy of capecitabine (bulk) in all solvent systems.

METHOD 1 0.1 N NaOH			
Sample No	**Concentration of capecitabine (µg/ml)**		**% Recovery**
	Theoretical	Experimental	
1	2	2	100
2	4	3.9	98.7
3	6	6	100
4	8	8.04	100.5
5	10	9.97	99.7
METHOD II 0.1 N HCl			
	Theoretical	Experimental	
1	2	2.01	100.5
2	4	4.02	100.5
3	6	5.9	99.2
4	8	8.0	100
5	10	10.2	100.2
METHOD III Methanol:Water (1:3)			
	Theoretical	Experimental	
1	2	2.02	101.1
2	4	3.9	99.4
3	6	6.03	100.5
4	8	8.04	100.5
5	10	10.06	100.6

The % recovery of capecitabine in formulations was found to be satisfactory with % RSD values of 1.924, 1.804, 1.691, for 0.1N NaOH, 0.1N HCl and Methanol: Water (1:3) solvent systems respectively which were within the acceptance limit. The results suggest that proposed methods were accurate in estimation. The data were shown in table 5.

Table 5: Data showing recovery studies of capecitabine (formulations) in all solvent systems.

METHOD I 0.1 N NaOH					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	4	40	13.9	99.2 ± 1.909	1.924
	2	20	11.8	98.7 ± 1.779	1.802
METHOD II 0.1 N HCl					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	4	40	14.03	100.2 ± 1.808	1.804
	2	20	12.03	100.3 ± 2.219	1.215
METHOD III Methanol:Water (1:3)					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	4	40	14.1	100.9 ± 2.707	1.691
	2	20	12.1	101.1 ± 3.143	1.130

Based on the standard deviation of the response and the slope the limit of detection values for capecitabine were found to be 0.191 µg/ml, 0.48 µg/ml, 0.51 µg/ml and limit of quantitation were found to be 0.584 µg/ml, 1.46 µg/ml, 1.54 µg/ml for 0.1N NaOH, 0.1N HCl, and Methanol:Water (1:3) respectively. The data were shown in table 6.

Table 6: Data showing LOD/LOQ of capecitabine in all solvent systems.

METHOD I 0.1 N NaoH		
	Mean ± SD	**SEM**
Limit of detection	0.191 ± 0.046	0.026
Limit of quantitation	0.584 ± 0.145	0.084
METHOD II 0.1 N HCl		
	Mean ± SD	**SEM**
Limit of detection	0.48 ± 0.047	0.027
Limit of quantitation	1.46 ± 0.148	0.084
METHOD III Methanol:Water (1:3)		
	Mean ± SD	**SEM**
Limit of detection	0.51 ± 0.134	0.072
Limit of quantitation	1.54 ± 0.384	0.224

The % RSD values of intra day and inter day precision for capecitabine in formulations were found to be less than 1.5 for 0.1N NaOH, 0.1N HCl, and Methanol: Water (1:3) respectively which were within the acceptance limit. The results suggest the proposed methods were precise and reproducible for the estimation. The data was shown in table 7.

Table 7: Data showing precision Intraday and Inter day trials with RSD values for capecitabine in all solvent systems.

METHOD I 0.1 N NaOH							
Trials	**Label claim (mg/tab)**	**Amount found (mg/tab)**		**% Label claim Mean ± SD**		**SEM**	**RSD**
Day-1	50	50.3 50.2 49.5		100.6 ± 1.246 100.4 ± 0.853 99.0 ± 0.642	**Intra day trials**	0.729 0.429 0.373	1.239 0.843 0.654
Day-2	50	50.1 49.2 49.6	**Intra day trials**	100.2 ± 1.474 98.4 ± 1.060 99.2 ± 1.385		0.851 0.611 0.860	1.473 1.062 1.388
Day-3	50	50.5 50.01 49.7		101.0 ± 0.692 100.02 ±0.961 99.4 ± 0.473		0.400 0.200 0.272	0.686 0.959 0.473
METHOD II 0.1 N HCl							
Day-1	50	50.4 50.3 50.2	**Intra day trials**	100.8 ± 1.381 100.6 ± 0.765 100.4 ± 1.024	**Intra day trials**	0.800 0.441 0.592	1.378 0.758 1.016
Day-2	50	50.4 50.2 50.3		100.8 ± 0.662 100.4 ± 1.204 100.6 ± 0.670		0.384 0.693 0.393	0.658 1.196 0.678
Day-3	50	50.2 49.9 49.6		100.4 ± 0.201 99.8 ± 1.386 99.2 ± 0.871		0.155 0.860 0.504	0.199 1.388 0.875
METHOD III Methanol:Water (1:3)							
Day-1	50	49.9 49.7 49.9	**Intra day trials**	99.8 ± 0.722 99.4 ± 0.791 99.8 ± 0.792	**Intra day trials**	0.416 0.458 0.458	0.718 0.796 0.803
Day-2	50	50.6 50.2 49.8		101.2± 1.155 100.4 ± 1.363 99.6 ± 0.757		0.665 0.913 0.437	1.156 1.575 0.760
Day-3	50	50.2 49.8 49.7		100.4 ± 1.22 99.6 ± 0.756 99.4 ± 1.151		0.705 0.437 0.664	1.213 0.754 1.156

Change in the λmax of ± 5nm to the actual λmax in robust analysis the % recovery of capecitabine was found to be significantly different which clearly indicates change in λmax of 5nm affected the method so proposed methods were not robust. Similarly change in the storage conditions during robust analysis, the %

recovery capecitabine is found to be significantly different which clearly indicates the storage condition is also affecting the method so proposed methods were not robust. The robust data were given in table 8,9.

Table 8: Data showing robustness of capecitabine at different wavelengths in all solvent systems.

METHOD	Conc (µg/ml)	Wave length	Amount found	Mean % ± SD	SEM	RSD
METHOD I	10	292 297 287	9.95 8.67 8.7	99.5 ±0.962 86.7 ±1.735 87.0 ±1.013	0.552 1.012 0.601	0.905 1.817 1.163
METHOD II	10	304 309 299	9.93 8.7 8.8	99.3 ±0.712 87 ± 1.507 88 ± 1.059	0.407 0.928 0.665	0.732 1.720 1.241
METHOD III	10	300 305 295	9.98 8.34 8.41	99.8 ±1.241 83.4 ±1.122 84.1 ±0.941	0.721 0.702 0.562	1.256 1.332 1.076

The % recovery of capecitabine in ruggedness analysis by different analyst and change of instrument viz., analyst-1; analyst-2 and instrument-1; instrument-2 shows the proposed methods were significantly rugged. The ruggedness data were shown in table 10, 11.

Table 10: Data showing ruggedness of capecitabine by different Analysts in all solvent systems.

METHOD	Conc (µg/ml)	Analyst	Amount found	Recovery± SD	SEM	RSD
METHOD I	10	Analyst 1 Analyst 2	10.01 9.94	100.1 ±0.54 99.4 ±0.3	0.223 0.122	0.539 0.301
METHOD II	10	Analyst 1 Analyst 2	9.99 10.05	99.9 ±0.75 100 .5±0.565	0.307 0.231	0.756 0.565
METHOD III	10	Analyst 1 Analyst 2	10.01 9.99	100.1 ±0.41 99.9 ±0.393	0.169 0.161	0.413 0.612

Table 11: Data showing ruggedness of capecitabine by using different Instruments in all solvent systems.

METHOD	Conc (µg/ml)	Instrument	Amount found	Recovery ± SD	SEM	RSD
METHOD I	10	Instrument 1 Instrument 2	9.92 10.01	99.2 ± 0.963 100.1 ±0.493	0.554 0.284	0.964 0.492
METHOD II	10	Instrument 1 Instrument 2	9.7 9.71	97.0± 0.709 97.1 ± 0.642	0.409 0.371	0.733 0.658
METHOD III	10	Instrument 1 Instrument 2	9.92 9.89	99.2 ± 1.249 98.9 ± 0.600	0.721 0.346	1.251 0.610

Robustness of capecitabine at refrigerated condition and room temperature.

	Trials	Label Claim (mg/tab)	REFREGERATED CONDITION						ROOM TEMPERATURE					
			Amount Found (mg/tab)		% Label Claim Mean ± SD		SEM	RSD	Amount Found (mg/tab)		% Label Claim Mean ± SD		SEM	RSD
Method-I	**Day-1**	50	49 49.1 48.8	**Intra day trials**	98 ± 0.341 98.2 ± 0.350 97.6 ± 0.352	**Intra day trials**	0.201 0.208 0.202	0.344 0.358 0.350	50.3 50.4 49.8	**Intra day trials**	100.6 ± 1.249 100.8 ± 0.850 99.6± 0.645	**Intra day trials**	0.727 0.424 0.373	1.233 0.842 0.654
	Day-2	50	48.8 48.4 48.5		97.6 ± 0.351 96.8 ± 0.251 99.0 ± 0.253		0.201 0.145 0.143	0.350 0.251 0.253	50.02 49.9 49.9		100.04 ± 1.474 99.8 ± 1.061 99.8 ± 1.383		0.852 0.611 0.850	1.474 1.062 1.382
	Day-3	50	48.6 49 49.1		97.2 ± 0.513 98.0 ± 0.251 98.2 ± 0.288		0.288 0.145 0.161	0.522 0.253 0.292	50.4 50.03 49.8		100.8 ± 0.692 100.06 ± 0.96 99.6 ± 0.473		0.400 0.201 0.273	0.684 0.959 0.473
Method-II	**Day-1**	50	49 49 49.1	**Intra day trials**	98 ± 0.603 98 ± 0.452 98.2 ± 0.360	**Intra day trials**	0.348 0.260 0.208	0.600 0.449 0.361	50.3 50.2 50.1	**Intra day trials**	100.6 ± 1.387 100.4 ± 0.763 100.2 ± 1.026	**Intra day trials**	0.800 0.441 0.592	1.378 0.758 1.016
	Day-2	50	49.1 49.2 49.1		98.2 ± 0.351 98.4 ± 0.451 98.2 ± 0.360		0.202 0.260 0.206	0.350 0.451 0.362	50.3 50.1 50.1		100.6 ± 0.665 100.2 ± 1.201 100.2 ± 0.681		0.384 0.693 0.393	0.658 1.196 0.678
	Day-3	50	49.4 49 49.1		98.8 ± 0.450 98 ± 0.556 98.2 ± 0.360		0.260 0.321 0.20	0.453 0.560 0.363	50.1 49.9 49.8		100.2 ± 0.200 99.8 ± 1.386 99.6 ± 0.875		0.155 0.860 0.504	0.199 1.388 0.873
Method-III	**Day-1**	50	49 49.1 49.2	**Intra day trials**	98 ±0.230 98.2± 0.550 98.4 ± 0.472	**Intra day trials**	0.133 0.318 0.272	0.229 0.548 0.471	50.1 49.7 49.8	**Intra day trials**	100.2 ± 0.722 99.4 ± 0.794 99.6 ± 0.793	**Intra day trials**	0.416 0.458 0.458	0.718 0.796 0.804
	Day-2	50	49 49 49.1		98 ± 0.472 98 ± 0.400 98.2 ± 0.458		0.272 0.230 0.264	0.474 0.4020.462	50.4 50.2 49.8		100.8± 1.153 100.4 ± 1.365 99.6 ± 0.471		0.665 0.913 0.788	1.156 1.575 1.369
	Day-3	50	49 49 49.1		98 ± 0.385 98 ± 0.655 98.2 ± 0.556		0.176 0.378 0.324	0.307 0.661 0.562	50.2 49.8 49.8		100.4 ± 1.22 99.6 ± 0.757 99.6 ± 1.150		0.705 0.437 0.664	1.212 0.754 1.156

CONCLUSION

The proposed UV spectrophotometric methods were found to be simple, rapid, accurate, precise and economic. From the above data it was observed that all validation parameters meet the predetermined acceptance criteria and validated in terms of linearity, accuracy, precision, reproducibility, robustness, and ruggedness as per the ICH guidelines. Thus it has been concluded that the proposed methods were validated for the analysis of capecitabine in bulk and its formulations.

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