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Research Article

Development and Validation of Simple UV Spectroscopic Method for the Quantification of Capecitabine in Its Pure Form

Vachan Y, Y. Anand Kumar*

Department of Pharmaceutics, V.L. College of Pharmacy, Raichur, Karnataka, India

ABSTRACT

The present research was aimed to study two solvent blends comprising Methanol: 1%Orthophosphoric acid (OPA) (77.5:22.5) and Methanol: pH 6.8 Buffer (1:1) for the quantification of model anticancer drug Capecitabine (CAP) in pure sample by validating simple UV spectroscopic method. A precise, accurate, robust simple UV method was developed under standard conditions and underwent rigorous statistical validation guided by the valuable ICHQ2R1 guidelines, affirming their effectiveness as a successful analytical tool for the quantification of CAP in pure sample. A linear relationship was found between 4 to 20 µg/mL for Solvent A and 5 to 28 µg/mL for Solvent B with correlation coefficient R^2 was 0.9999 and 0.9999 respectively. The precision study indicates the method was reproducible, accurate with % RSD values less than 2% and highly robust. The results conclude that the developed method can be used for routine analysis of bulk and formulations containing CAP.

Keywords: Capecitabine; UV spectroscopy; Validation; Accuracy; Precision**ARTICLE INFO:** Received 29 July 2026; Review Complete 15 August 2026; Accepted 10 Sept. 2026; Available online 15 Oct. 2026**Cite this article as:**Vachan Y, Y. Anand Kumar, Department of Pharmaceutics, V.L. College of Pharmacy, Raichur, Karnataka, India, Asian Journal of Pharmaceutical Research and Development. 2026; 14(5): 49-54 DOI: <http://dx.doi.org/10.22270/ajprd.v14i5.1867>

*Address for Correspondence:

Dr.Y. Anand Kumar, Professor and Head, Department of Pharmaceutics, V.L. College of Pharmacy, Raichur-Karnataka, India

INTRODUCTION

Capecitabine (CAP) is a fluoropyrimidine carbamate with antineoplastic activity, indicated for the treatment of metastatic breast cancer and colorectal cancer. CAP is an orally administered systemic prodrug that belongs to the class of organic compounds known as glycosylamines, undergoes selective activation within tumor tissues to its cytotoxic metabolite, 5-fluorouracil (5-FU). This conversion is mediated by thymidine phosphorylase, an enzyme present at higher concentrations in many tumors than in normal tissues or plasma.

Once formed, 5-fluorouracil is further metabolized in both normal and tumor cells into two active metabolites: 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites induce cytotoxicity through two distinct mechanisms. First, FdUMP forms a covalently bound ternary complex with thymidylate synthase (TS) and the folate cofactor N^5, N^{10} -methylene tetrahydrofolate, thereby inhibiting the conversion of 2'-deoxyuridylate to thymidylate.

Thymidylate is an essential precursor of thymidine triphosphate, which is required for DNA synthesis; its depletion consequently inhibits DNA replication and cell division. Second, FUTP can be erroneously incorporated into RNA in place of uridine triphosphate (UTP) during transcription. This misincorporation disrupts RNA processing and protein synthesis, leading to the production of defective RNA and further contributing to cellular injury¹⁻⁸. Chemically, CAP is designated as pentyl N-{1-[(2R, 3R, 4S, 5R)-3, 4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxo-1,2-dihydropyrimidin} (figure 1).

A few methods were reported in the literature for the quantification of CAP in bulk and pharmaceutical formulations viz., UV spectroscopic⁹⁻¹¹, First order derivative spectroscopic¹², Fluorimetric¹³, HPLC¹⁴, HPTLC¹⁵ and RP-HPLC¹⁶⁻²⁰. Hence in the present work it was aimed to develop and validate accurate, precise, simple and rapid UV spectroscopic method for the quantification of CAP in pure form as per ICH guidelines. This approach provides a cost effective and simplifying precise dosage determination.

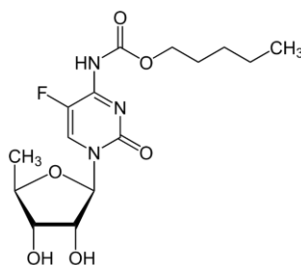


Figure 1 Chemical structure of CAP

EXPERIMENTAL

Materials: A gift sample of Capecitabine (CAP) was provided by Shilpa Medicare Ltd., Raichur, Karnataka, India, for analytical purposes. Analytical grade methanol was used as the solvent and double-distilled water was obtained from a Qualigens distillation unit. Other chemicals, including orthophosphoric acid (H_3PO_4), sodium dihydrogen orthophosphate (NaH_2PO_4), and sodium hydroxide (NaOH), all of analytical reagent grade, were procured from S.D. Fine Chemicals, Mumbai, India. The use of high quality reagents and solvents ensured the reliability and accuracy of the analytical procedures.

Instruments and software: Spectrophotometric measurements were performed using a Hitachi 1900 double-beam UV-Vis spectrophotometer with a spectral bandwidth of 1 nm and a wavelength accuracy of ± 0.5 nm. Data acquisition and analysis were conducted using LabSolutions software. Absorbance measurements were obtained using 1 cm pathlength quartz cells. The pH of the buffer solutions was measured using a Globi Instruments digital pH meter. Sample weights were determined using a Wensar analytical balance. A temperature controlled, high precision water bath and an ultrasonicator were used to facilitate dissolution of the active pharmaceutical ingredient (API) under investigation.

Screening of the solvents: A range of solvent systems, including organic solvents, aqueous solvents at varied pH, distilled water, alone and their combinations at various ratios were screened for solvent selection. Based on solubility and stability considerations, two optimal solvent blends were identified for the quantification of CAP viz., Solvent A comprising Methanol: 1% OPA (77.5:22.5), and Solvent B comprising Methanol: Phosphate buffer pH 6.8 (50:50).

Standard stock solution preparation: 25 mg of CAP was dissolved in 10 ml Solvent A by giving good shake and 10 min sonication to obtain the final concentration of 1000 $\mu\text{g/mL}$. Similarly 1000 $\mu\text{g/mL}$ stock solution of CAP was prepared in Solvent B.

Standard working solution preparation: 2.5 mL of each stock solution was transferred into two 25 mL volumetric flask, make up the volume with Solvent A and Solvent B separately to achieve 100 $\mu\text{g/mL}$ solutions.

METHODS

Absorption maxima (λ max) determination: 1 mL standard working solution of each solvent systems were transferred separately in two 10 mL volumetric flask, make up the volume with Solvent A and Solvent B separately to achieve 10 $\mu\text{g/mL}$ solutions. Each solution was scanned at 200 to 400 nm and record absorption maxima with characteristic peak at standard wavelength.

Method validation

The International Council for Harmonisation (ICH) of technical requirements for pharmaceuticals for human use established standards for the validation of the analytical procedures utilized in this investigation.

Linearity and range: The linearity is the ability of analytical procedure to produce test results, which are proportional to the concentration (amount) of analyte in samples within a given concentration range. The working solutions of CAP were appropriately diluted to determine the concentration range (1 to 60 $\mu\text{g/mL}$) linearity (4 to 20 and 5 to 28 $\mu\text{g/mL}$) and were analyzed in two solvents blends at chosen wavelengths (241 nm) by linear regression.

Accuracy: The percentage of an analyte recovered from a recognized brought quantity was studied at 3 concentration levels (50, 100, and 150 %) and the percentage relative deviation (RSD) of the patterns recovered was used to display the outcomes.

Precision: The Intraday and Interday precisions also referred as repeatability and intermediate accuracy, respectively were used to assess the precision of two solvent blend used. Precision of proposed analytical method was carried out at two known concentrations (six replicates) viz., 14, 20 $\mu\text{g/mL}$ for Solvent A and 16, 20 $\mu\text{g/mL}$ for Solvent B. The experiment was conducted on the same day and for the next three days for both Intraday and Interday precision, analysing freshly made solutions at concentrations of 14, 20 $\mu\text{g/mL}$ for Solvent A and 16, 20 $\mu\text{g/mL}$. For analysis the standard CAP solutions were appropriately diluted with solvent blends the CAP working standard solution in Solvent A and Solvent B under the study and express the results in terms of % RSD. This meticulous approach ensures a comprehensive evaluation of the precision of the analytical method, providing confidence in the reliability and consistency of the results obtained for the stated concentrations of CAP in the tested solutions.

Robustness: The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in analytical situations. The samples have been subjected to evaluation below changed situations, which include detection wavelength (± 2 nm and ± 5). The resulting results on machine suitability parameters have been intently monitored.

LOD and LOQ: Limit of detection (LOD) is the lowest amount of an analyte detected in a sample and Limit of quantitation (LOQ) is the lowest amounts of an analyte quantified were decided for the newly evolved approach following ICH pointers. The calculations for LOQ and LOD were executed using the equations $LOD = 3.3 \times SD/S$; $LOQ = 10 \times SD/S$ respectively, where "s" stands for the mean of the slope of the calibration plot and "SD" is the standard deviation of the regression line at the y-intercept.

RESULTS AND DISCUSSION

Absorption maxima, Linearity and range: Two characteristic absorption peaks were observed at 241

nm for both the Solvent A and Solvent B (figure 2) which are in corroborate with the literature. The linearity curve was constructed at 241 nm for Solvent A at CAP concentrations of 4, 6, 10, 14, 18 and 20 $\mu\text{g/mL}$ and 5, 10, 16, 20, 24 and 28 $\mu\text{g/mL}$ for Solvent B at 241 nm (table 1, figure 3). The molar absorptivity of CAP was found to be 0.04 and 0.02 $\text{lit/mol}\cdot\text{cm}$ for Solvent A and Solvent B; Sandell's sensitivity was 0.023 $\mu\text{g/cm}^2$ and 0.048 $\mu\text{g/cm}^2$ for Solvent A and Solvent B respectively. A linear relationship was found between 4 to 20 $\mu\text{g/mL}$ for Solvent A and 5 to 28 $\mu\text{g/mL}$ for Solvent B) with correlation coefficient R^2 was 0.9999 and 0.9999 respectively. The regression equation for linearity curve was $Y = 0.04015 \cdot X - 0.0008462$ for Solvent A and $Y = 0.01995 \cdot X - 0.0003390$ for Solvent B. The results shows the validity of Beer's law with intercept response $< 2\%$ calculated by the least square method indicating functional linearity between the concentration of CAP and the absorbance.

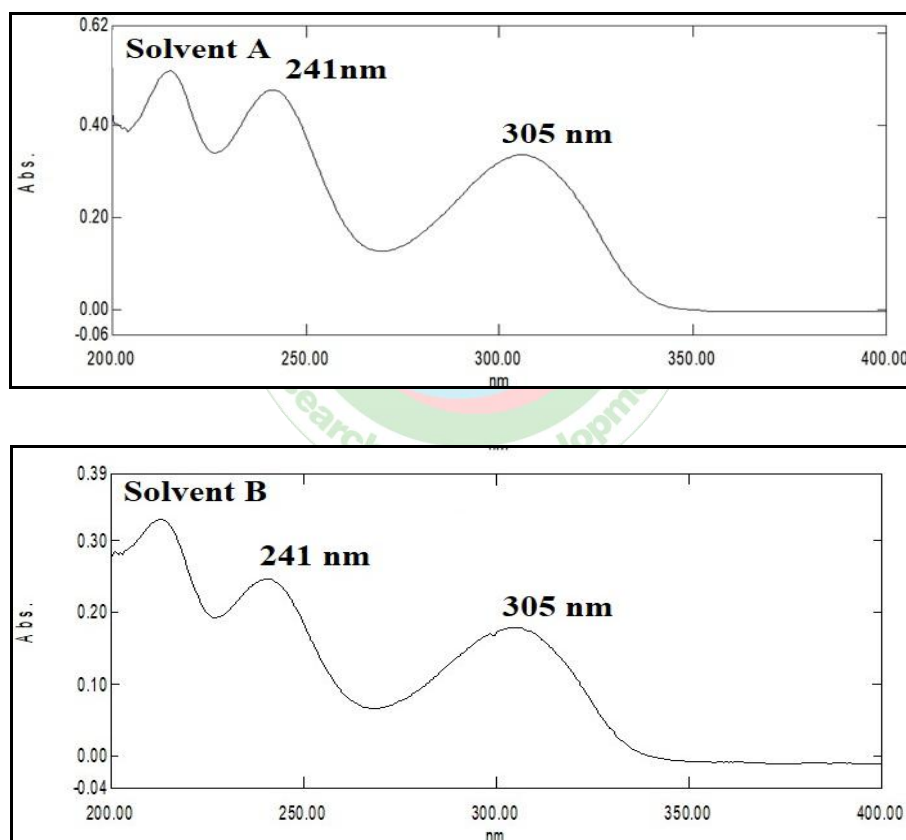


Figure 2: Absorption maxima of CAP in Solvent A and Solvent B

Table 1: Linearity curve data of CAP in Solvent A and Solvent B

Solvent A			Solvent B		
Concentration $\mu\text{g/ml}$	Absorbance Mean $\pm$ SD	% RSD	Concentration $\mu\text{g/ml}$	Absorbance Mean $\pm$ SD	% RSD
4	0.160 $\pm$ 0.0005	0.312	5	0.1018 $\pm$ 0.0005	0.4914
6	0.240 $\pm$ 0.0005	0.2081	10	0.2053 $\pm$ 0.0005	0.2436
10	0.401 $\pm$ 0.0008	0.2036	16	0.3223 $\pm$ 0.0005	0.1552
14	0.561 $\pm$ 0.0012	0.2244	20	0.4018 $\pm$ 0.0005	0.1245
18	0.720 $\pm$ 0.0005	0.0694	24	0.4813 $\pm$ 0.0005	0.1039
20	0.804 $\pm$ 0.0019	0.2381	28	0.5628 $\pm$ 0.0005	0.0888

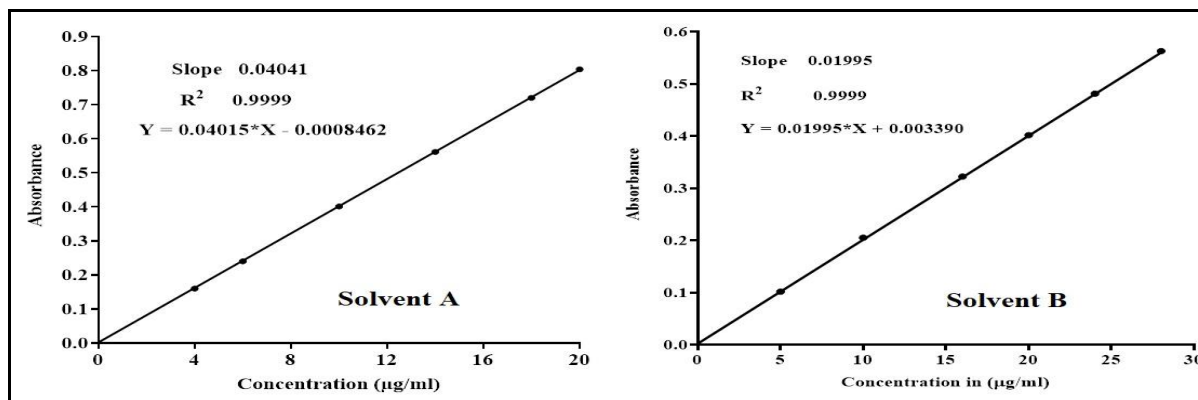


Figure 3: Linearity curve of CAP in Solvent A and Solvent B

LOD and LOQ

The quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured in terms of LOD and LOQ. Based on standard deviation of the response and slope, the LOD values for CAP for the solvent blends found to be 0.0743 µg/ml, 0.0551 µg/ml and LOQ values found to be 0.0827 µg/ml, 0.2506 µg/ml with % RSD values less than 2.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The precision data (table 2) in terms of repeatability, intra and inter day of the proposed solvent blends were studied for two fixed amounts (14, 20 µg/mL for Solvent A and 16, 20 µg/mL for Solvent B) with six replicates, the % RSD was less than 2 indicating the solvent blends under the study were precise and reproducible over the period of 72 hr.

Accuracy

The accuracy of the solvent blends under the study were done by standard addition method, which involves addition of three levels viz., 50%, 100%, and 150% standard CAP to the sample solution, and the recovery of the CAP was evaluated. The mean percentage recovery of CAP in Solvent A and Solvent B were within the acceptable range of 100.21-101.95 with % RSD less than 2 indicating that the solvent blends under the study were accurate (table 3).

Robustness

In robustness the experimental variations viz., change in λ max of ± 5 nm and ± 2 nm to the actual λ max was studied on Solvent A and Solvent B (table 4). The result shows that there was no statistically significant difference in the % RSD (less than 2) for the solvent blends under the study, indicate both proposed solvents were highly robust.

Stability

The stability of proposed solvent blends was studied and % recovery was found to be within the acceptable limits indicate stable at Room temperature and stored temperature viz., 40°C, 4-8°C over the period of 72 hr.

Table 2: Precision data of CAP in Solvent A and Solvent B

Precision	Solvent A				Solvent B			
	Labelled Claim µg/ml	Amount recovery µg/ml	Mean % Recovery $\pm$ SD n=6	% RSD	Labelled claim µg/ml	Amount recovery µg/ml	Mean % Recovery $\pm$ SD n=6	% RSD
	14	14.08	100.6 $\pm$ 0.387	0.385	16	16.09	100.6 $\pm$ 0.233	0.232
	20	20.03	100.1 $\pm$ 0.186	0.186	20	20.02	100.2 $\pm$ 0.137	0.137
Intraday Precision								
24 hr	14	14.1	100.7 $\pm$ 0.176	0.175	16	16.05	100.3 $\pm$ 0.179	0.178
24 hr	20	20.02	100.2 $\pm$ 0.141	0.144	20	20	100.0 $\pm$ 0.144	0.144
Interday Precision								
0 hr	14	14.12	100.8 $\pm$ 0.081	0.080	16	16.05	100.3 $\pm$ 0.179	0.178
	20	20.02	100.2 $\pm$ 0.141	0.144	20	20.05	100.3 $\pm$ 0.289	0.287
24 hr	14	14.12	100.8 $\pm$ 0.081	0.080	16	16.05	100.2 $\pm$ 0.179	0.178
	20	20.02	100.2 $\pm$ 0.141	0.144	20	20.05	100.3 $\pm$ 0.289	0.287
48 hr	14	14.05	100.4 $\pm$ 0.087	0.083	16	16.05	100.3 $\pm$ 0.179	0.178
	20	20.02	100.2 $\pm$ 0.141	0.144	20	20.05	100.3 $\pm$ 0.288	0.287
72 hr	14	14.1	100.7 $\pm$ 0.176	0.175	16	16.05	100.3 $\pm$ 0.179	0.178
	20	20.02	100.2 $\pm$ 0.141	0.144	20	20	100.0 $\pm$ 0.144	0.144

Table 3: Accuracy data of CAP in Solvent A and Solvent B

Solvent A					
Labelled Claim µg/ml	% Addition	Amount added	Amount Recovery µg/ml	Mean % Recovery ± SD N=6	% RSD
14	50	7	17.11	100.64±0.0173	0.0172
14	100	14	28.15	100.53±0.0980	0.0982
14	150	21	31.16	100.51±0.0152	0.0152
20	50	10	30.07	100.23±0.0352	0.0349
20	100	20	40.08	100.71±0.0100	0.0099
20	150	30	50.02	100.04±0.0101	0.0097
Solvent B					
16	50	8	24.34	101.41±0.0152	0.0150
16	100	16	32.54	101.68±0.0208	0.0204
16	150	24	40.78	101.95±0.0208	0.0204
20	50	10	30.36	101.20±0.0152	0.0150
20	100	20	40.49	101.23±0.0103	0.0099
20	150	30	50.22	100.44±0.0152	0.0150

Table 4: Robustness data for CAP in Solvent A and Solvent B

λ max nm	Labelled claim µg/ml	Amount recovery µg/ml	Mean % Recovery ± SD N=6	% RSD
± 2	Solvent A			
Actual 241	14	14.10	100.7±0.1762	0.1750
	20	20.02	100.1±0.1413	0.1441
239	14	14.12	100.8±0.0808	0.0801
	20	20.02	100.1±0.1413	0.1441
243	14	14.12	100.8±0.0808	0.0801
	20	20.02	100.1±0.1413	0.1441
± 5				
Actual 241	14	14.05	100.4±0.0866	0.0826
	20	20.02	100.1±0.1413	0.1441
236	14	14.1	100.7±0.1762	0.1750
	20	20.02	100.1±0.1413	0.1441
246	14	14.12	100.8±0.0808	0.0801
	20	20.02	100.1±0.1413	0.1441
± 2	Solvent B			
Actual 241	16	16.05	100.4±0.1790	0.1782
	20	20	100.0±0.1443	0.1442
239	16	16.09	100.6±0.2334	0.2320
	20	20.02	100.1±0.1369	0.1368
243	16	16.05	100.4±0.1790	0.1782
	20	20.05	100.2±0.2887	0.2875
± 5				
Actual 241	16	16.05	100.4±0.1790	0.1782
	20	20.05	100.2±0.2887	0.2875
236	16	16.05	100.4±0.1790	0.1782
	20	20.05	100.2±0.2887	0.2875
246	16	16.05	100.4±0.1790	0.1782
	20	20	100.0±0.1443	0.1442

CONCLUSION

The proposed UV spectrophotometric methods were simple, rapid, accurate, precise, and economical. All validation parameters met the acceptance criteria in accordance with ICH guidelines, confirming the suitability of the methods for the analysis of Capecitabine in its pure form.

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DISCLOSURE OF CONFLICT OF INTEREST

No conflict of interest to be disclosed.

AUTHOR'S CONTRIBUTION

The collaborative efforts of all authors have successfully led to the development of a UV spectroscopic method for estimating Capecitabine in pure form. Vachan Y under the mentorship of Y Anand Kumar conducted the development and validation of the UV spectroscopic technique for Capecitabine. Y. Anand Kumar provided guidance in crafting the manuscript and structuring the research paper in accordance with the journal's guidelines.

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