

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF CAPECITABINE IN API AND FORMULATION

*E. Ramakrishna Goud, R. Nageswara Rao, S. Sulakshana, V. Ravisankar, L. Siva Sankar Reddy

Department of Pharmaceutical Analysis and Quality Assurance, Creative Educational Society's College of Pharmacy, JNTUA, Kurnool, Andhra Pradesh.

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*Corresponding Author

E. Ramakrishna Goud

Department of
Pharmaceutical Analysis
and Quality Assurance,
Creative Educational
Society's College of
Pharmacy, JNTUA,
Kurnool, Andhra Pradesh.

ABSTRACT

An effort has been made to develop a simple, accurate method to estimate capecitabine in API and pharmaceutical preparation and to validate the method, according to ICH guidelines. The absorbance maxima was recorded at a wavelength of 303 nm for phosphate buffer pH 7.4 which is shown in Beers law range was confirmed by linear curve of capecitabine, shown Linearity for capecitabine at concentration range of 10-30 μ g/ml. From the above studies the optical characteristics such as linearity range (10-30 μ g/ml), for phosphate buffer pH 7.4 of correlation coefficient (0.999), slope (0.0266) and intercept (0.0025) were calculated and results were found to be satisfactory. Quantitative data subjected to statistical analysis. The % RSD values < 2 indicate the precision of methodology. The accuracy was confirmed by recovery studies by adding a known amount of pure

drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 98% - 102%. The values are given in recovery was confirmed and shown.

KEYWORDS: UV-Spectrophotometry, Capecitabine, Validation.

INTRODUCTION

Capecitabine is chemically Pentyl[1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H-pyrimidin-4-yl] amino methanoate. Its Molecular formula is C₁₅H₂₂FN₃O₆ and possessing a molecular weight of 359.35. It is Soluble in aqueous solvents and insoluble in

organic solvents. Capecitabine is used as an anti-cancer drug. The structural formula of Capecitabine is shown in fig-1. Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development in the recent era. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeia. Only few method are available like UV, Visible and RP-HPLC methods. Hence, it is essential to develop newer analytical methods, which are accurate, precise, and specific, linear, simple and rapid. From the extensive literature survey, it was revealed that there were a very few methods reported for pharmaceutical dosage forms. Therefore, here an attempt was made to develop simple, cost effective and accurate method.

MATERIALS AND METHODS

Materials

Capecitabine obtained as a gift sample from, Celon laboratories, HYD, all the chemicals used are of analytical grade.

Instrument

LAB INDIA –Double Beam UV-VIS Spectrophotometer3000+ with a pair of 1.0 cm matched quartz cells was used for the measurement of absorbance.

METHODOLOGY

SELECTION OF SOLVENT

The solubility of capecitabine was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test was carried out in different polar and nonpolar solvents from the solubility studies, phosphate buffer pH 7.4 were selected as suitable solvents for the proposed method.

PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution was prepared by dissolving, accurately weighed 10 mg of CAPECITABINE in phosphate buffer pH7.4 and the volume was made up to 10 ml with phosphate buffer pH7.4 in 10 ml volumetric flask (1° Stock solution, 1000 µg / ml).

DETERMINATION OF ABSORBANCE MAXIMA (λ_{max})

10 ml of primary stock solution was diluted to 100 ml with phosphate buffer pH7.4 (secondary Stock solution, 100 µg / ml). 1 ml of secondary Stock solution was taken in 10 ml standard volumetric flask diluted to 10 ml with phosphate buffer pH7.4 to get the

concentration of 10 μ g/ml. The absorbance of resulting solution was measured against respective blank solution (phosphate buffer pH 7.4) in the UV region of 200-400 nm, which shows maximum absorbance at 303 nm.

ANALYSIS OF MARKETED FORMULATION

10 tablets of CAPECITABINE were weighed, pulverized and the powder equivalent to 100 mg of CAPECITABINE was weighed accurately and transferred into a 100 ml standard volumetric flask. This solution was sonicated for 10 minutes. After sonication the solution was centrifuge at 100 rpm for 10 minutes the solution was filtered through Whatmann filter paper number 41. 0.2 ml of above solution was diluted to 10 ml with phosphate buffer pH 7.4 to obtain a solution of 20 μ g / ml. same concentration was repeated three times.

METHOD VALIDATION

ACCURACY

Accuracy is the closeness to the true value. To study the accuracy, 10 tablets of Capecitabine were taken, and the powder was used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution (80%, 100%, 120% μ g/ml) to the sample at 3 different concentration levels and results were presented in Table No: 3.

PRECISION

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples shown in Table 5 & 6.

Intra and inter-day precision

A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed and was shown in table no: 5. Intra-day precision was determined by analyzing Capecitabine for three times in the same day at 303 nm. Inter-day precision was determined by analyzing the drug daily once for three days at 303 nm.

LINEARITY

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Capecitabine were transferred into a series of 10 mL standard flasks and volumes were made upto mark with phosphate buffer pH 7.4. Five different concentrations were prepared in the range of 10-30 μ g/mL and the absorbances were measured at 303 nm

against solvent (phosphate buffer pH 7.4) blank and the absorbance values were shown in. The obtained absorbance values are plotted against the concentration of Capecitabine to get the calibration graph and were represented as the concentration of the unknown sample was determined from the calibration graph. The regression equation and correlation coefficient were determined and are given in.

LIMIT OF DETECTION (LOD)

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. It is calculated from the formula,

$$\text{LOD} = 3.3 \sigma / S$$

LIMIT OF QUANTITATION (LOQ)

Based on the LOD strength, the LOQ values were calculated by multiplication with three times. The quantitation limit (QL) is calculated from,

$$\text{LOQ} = 10 \sigma / S$$

LIMIT OF DETECTION

LIMIT OF QUANTIFICATION

The parameter LOQ was determined on the basis of response and slope of regression equation

RESULTS

An effort has been made to develop a simple, accurate method to estimate Capecitabine in bulk and pharmaceutical preparation and to validate the method, according to ICH Q2 (R1) guidelines.

The absorbance maxima was recorded at wavelength of 303 nm which is shown in Fig: 2. Beers law range was confirmed by linear curve of Capecitabine, shown in Fig: 3. Linearity for Capecitabine is shown at concentration range of 10 -30 $\mu\text{g} / \text{ml}$.

From the above studies the optical characteristics such as linearity range (10-30 $\mu\text{g} / \text{ml}$), correlation coefficient (0.9998), slope (0.0266) and intercept (0.0025) were calculated and results were found to be satisfactory. Quantitative data subjected to statistical analysis. The % RSD values < 2 indicate the precision of methodology.

The accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 99.27%. The values are given in recovery was confirmed and shown in the table: 4. the parameter LOD and LOQ was found to be 0.20215 $\mu\text{g/ml}$ 0.61259 $\mu\text{g/ml}$.

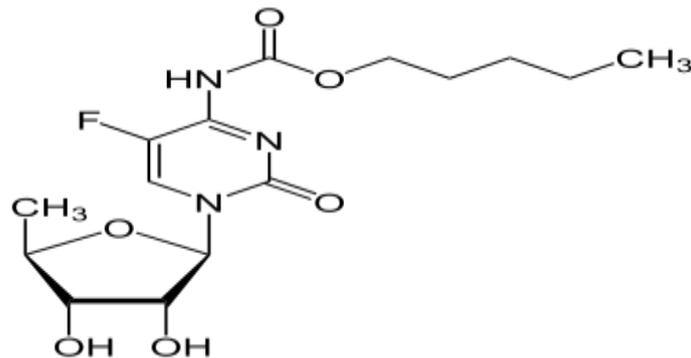


Figure1: Chemical structure of Capecitabine

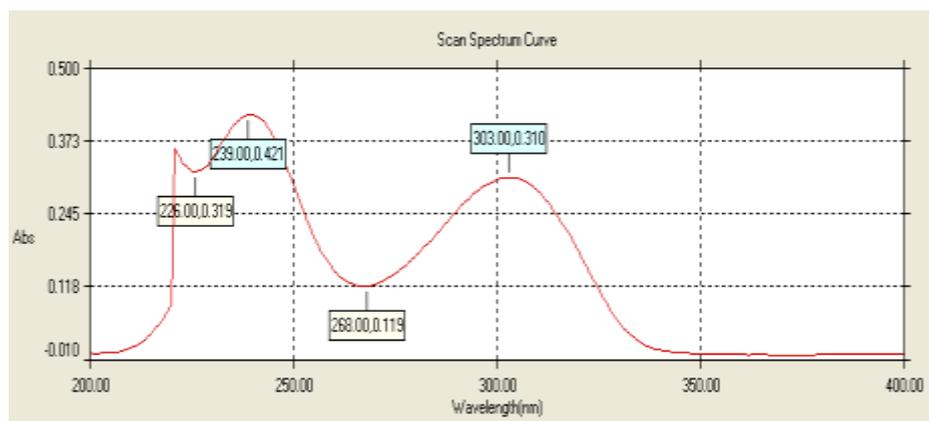


Figure 2: λ -max of Capecitabine

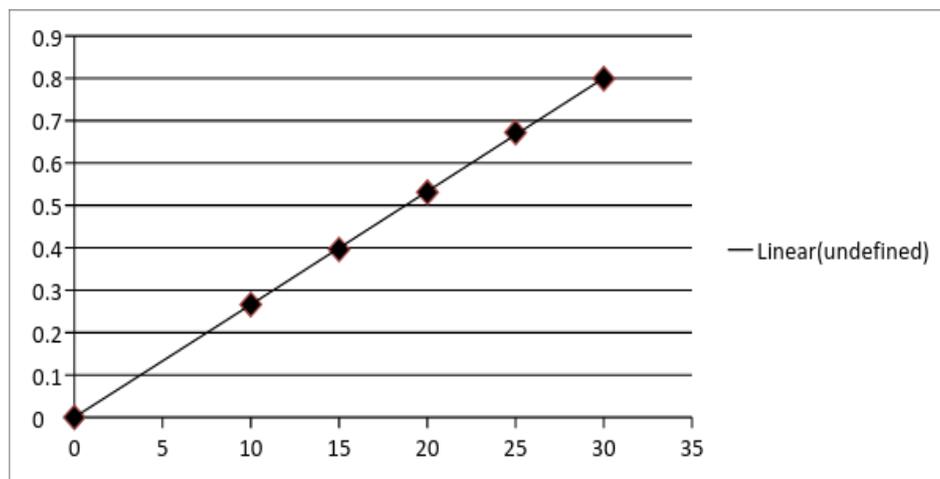


Figure 3: Calibration Curve of Capecitabine

Table No 1: Calibration Curve

S.No	Concentration	Absorbance
1	10	0.256
2	15	0.396
3	20	0.535
4	25	0.672
5	30	0.797

Table No: 2 Optical Characteristics of Capecitabine

Values	Parameters
303	λ_{\max} (nm)
10-30	Beer's law limits ($\mu\text{g/ml}$)
$Y = 0.0266x + 0.0025$	Regression equation (Y^*)
0.0266	Slope (b)
0.0025	Intercept (a)
0.9998	Correlation coefficient (r^2)
< 2%	% RSD
0.2021	Limit Of Detection ($\mu\text{g/ml}$)
0.6125	Limit Of Quantitation ($\mu\text{g/ml}$)

Table No 3: Assay of Capecitabine

S.No	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3) Mean \pm SD	Assay	%RSD
1	Capecitabine	500mg	19.8558 \pm 0.0005	99.27%	0.00005

Table No 4: Determination of Accuracy Results For Capecitabine

S.No	% added	Amount (μg)	Amount Added (μg)	Amount found (μg)	Amount recovered (μg)	% recovery (x)	SD	%RSD	
1		20	16	35.80	15.80	99.35	0.00012	0.00012	
2	80%			36.10	16.10				
3				35.58	15.58				
1			20	20	39.34				19.34
2	100%				39.90				19.90
3					39.49				19.49
1			24	24	44.64				24.64
2	120%				43.21				23.21
3					45.01				25.01

Table No 5: Intraday Precision Results Capecitabine

S. No	Concentration ($\mu\text{g/ml}$)	Amt. found ($\mu\text{g/ml}$)	Percentage (x) (%)	Average	SD	%RSD
1.	20	20.16	100.84			
2.	20	19.75	98.77			
3.	20	20.31	101.59	99.30	0.0064	0.0064
4.	20	19.41	97.08			
5.	20	19.64	98.21			
6.	20	19.86	99.34			

Table No 6: Interday Precision Results Capecitabine

S. No	Concentration (µg / ml)	Amt. found (µg / ml)	Percentage (x) (%)	Average	SD	%RSD
1.	20	19.90	101.25			
2.	20	20.16	102.12			
3.	20	20.39	100.87	99.56	0.00005	0.00005
4.	20	19.45	101.75			
5.	20	19.90	101.25			
6.	20	19.64	102.5			

CONCLUSION

All the above parameters combined with simplicity and ease of operation ensure that the application of proposed method from UV Spectrophotometric method for estimation of Capecitabine was found to be useful with high accuracy, precision. It can be used for routine analysis of capecitabine in Api and pharmaceutical preparation.

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