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Validated UV and visible spectrophotometric method for the estimation of capecitabine – A cancer drug

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ABSTRACT

To develop and validate a simple, rapid, accurate and economical UV and visible Spectrophotometric method for determination of capecitabine in bulk and its formulation product. UV and Visible Spectrophotometric method was performed by using UV/Vis double beam spectrophotometer with spectral band width of 1 nm and 1.0 cm matched quartz cells and glass cells were used for UV and Visible regions respectively. Methanol was used as solvent and the maximum absorbance of Capecitabine was observed in methanol at 308nm in UV region and 616nm in visible region respectively. The linear calibration range was found to be 5µg/mL to 30µg/mL in UV and 10µg to 100µg/mL in visible region respectively. The correlation coefficient (R^2) is 0.9985 and the regression equation is y=0.0329x-0.747 in UV region and correlation coefficient (R^2) is 0.9989 and the regression equation is y=0.0081x+0.0356 in visible region respectively. The method was validated in terms of Linearity, Precision, Accuracy, Robustness, LOD and LOQ as per ICH guidelines.

Keywords: Capecitabine, UV- Visible Spectrophotometer, Validation.

INTRODUCTION

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity indicated for the treatment of metastatic breast cancer and colon cancer. It is an orally administered systemic prodrug. This compound belongs to the class of organic compounds known as glycosylamines. These are compounds consisting of an amine with a beta-N-glycosidic bond to a carbohydrate, thus forming a cyclic hemiaminal ether bond (alpha-amino ether).It is chemically pentyl N-{1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxo-1,2-dihydropyrimidin. It readily absorbed through the GI tract (~70%). Capecitabine is a prodrug that is selectively tumour-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase, an enzyme found in higher concentrations in many tumors compared to normal tissues or plasma. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumour cells. These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N5-10-methylenetetrahydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2'-deaxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, therefore a deficiency of this compound can inhibit cell division. Secondly, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis through the production of fraudulent RNA [1-5].

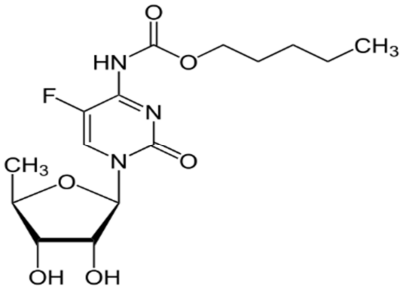

Fig 1: Structure of capecitabine [4]

MATERIALS AND METHODS

Instrument:
Lab India – T60, UV/Vis double beam spectrophotometer with spectral band width of 1 nm, wavelength accuracy of ± 0.3 nm and 1.0 cm matched quartz cells and glass cells were used for UV and Visible respectively for the method development of capecitabine.

Chemicals and Reagents:
All the reagents were of analytical grade. Methanol (Merck, Mumbai, India) was used. Capecitabine pure drug was obtained as a gift sample from MSN Laboratories Ltd, Hyderabad, India. Bromo cresol green is used as the visible reagent.

Selection of solvent:
Drug showed maximum absorbance in methanol and hence methanol was used as solvent for further preparation of solutions.

Selection of detection wavelength:
For UV region:
Drug solution of 30µg/mL was scanned over the range of 200-400nm in UV region. It was observed that the drug showed maximum absorbance at 308nm and hence 308nm was selected as the detection wavelength.

For Visible region:
Drug solution of 100µg/ml(by adding bromo cresol green reagent) was scanned over the range of 400-800nm in Visible region. It was observed that the drug showed maximum absorbance at 616nm and hence 616nm was selected as the detection wavelength.

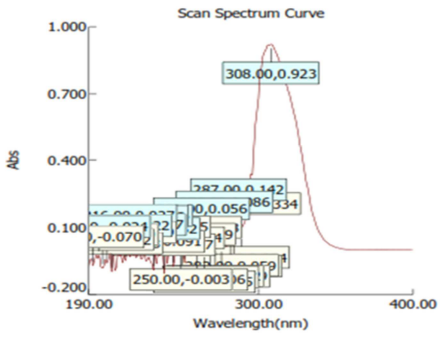

Fig 2: Capecitabine UV spectrum

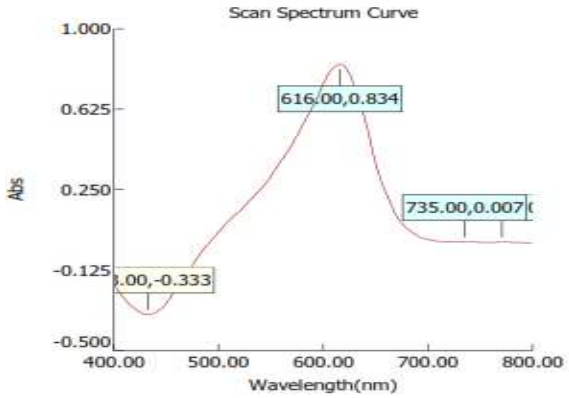

Fig 3: Capecitabine Visible spectrum

Preparation of Standard stock solution:
100mg of Capecitabine pure drug was accurately weighed and taken into a 100ml volumetric flask. About 20ml of methanol was added to it and sonicated for 15 minutes and then the volume was made up to 100ml with methanol to make 1000µg/ml stock solution. From the above solution 2.5ml was pipetted out into a 25ml volumetric flask and volume was made up to the mark with methanol to get 100µg/mL concentration is called working standard solution.

Preparation of Sample solutions:
20 tablets were weighed and finely powdered. Powder equivalent to 100mg of drug was weighed and taken into 100mL volumetric flask. About 20 ml of methanol was added to the flask and sonicated for 15mins and then the volume was made up to 100ml with methanol to make 1000µg/mL stock sample solution. Then 100 µg/mL working sample solution was prepared by pipetting 10ml of 1000µg/mL solution into 100ml volumetric flask and filling the remaining volume with methanol.

Preparation of dilutions for calibration curve construction:
For UV method:
Pipetted0.5,1,1.5,2,2.5 and 3mL solutions from working solution into 10 ml volumetric flasks. And fill the volume to mark with diluent. This gives dilutions of 5,10,15,20,25 and 30µg/ml solutions respectively.

For visible method:
Pipette out 2, 4, 6, 8 and 10 mL solutions from working solution into 10 ml volumetric flasks. Now 0.5 Ml of 1% bromo cresol green reagent was added and allowed to stand for 1 min to develop colour and fill the volume to mark with diluent. This gives dilutions of 20, 40, 60, 80 and 100µg/Ml solutions respectively.

Visible reaction mechanism of capecitabine:
Capecitabine is having an amino group in the molecular structure making it possible to form the ion-pair complexes with acidic dye namely bromocresol green (BCG). The ion-association complex or adduct (commonly known as ion pair, if two ions are involved) is a special form of molecular complex.

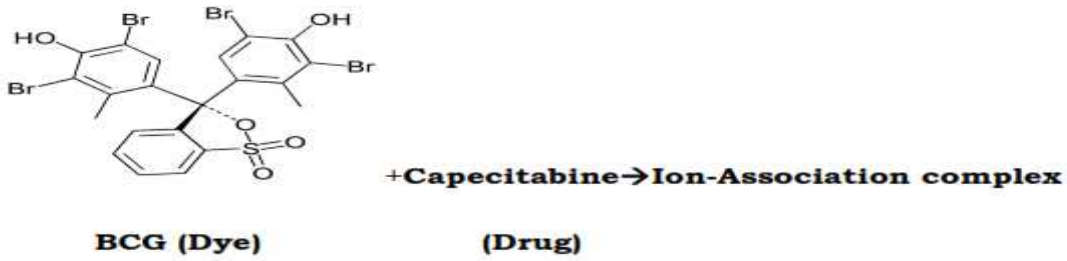

Fig: 4 Reaction mechanism of capecitabine

Method Validation [6-9]
Linearity and Range
The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. For the establishment of linearity, minimum of five concentrations are recommended by ICH guideline. The value of correlation co-efficient (r 2) should fall around 0.99. The regression equation and correlation coefficient were calculated and found to be within the required limits as per ICH guidelines. The results were shown in table no.1 and 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. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The results were indicated by % RSD which were shown in table no 3 and 4.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The evaluation of accuracy has got very prime importance as it deliberately force the method to extract the drug and impurities at higher and lower level. The results were shown in table no.5 and 6.

Limit of Detection
The detection limit 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.

LOD = 3.3 (SD / S)

Where, SD = the standard deviation of the response
S = the slope of the calibration curve

Limit of Quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precise.

LOQ = 10 (SD / S)

Where, SD = the standard deviation of the response
S = the slope of the calibration curve

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage the results were indicated by % RSD which were shown in table no: 9 and 10.

Optimized characteristics data

S. NO	Parameters	UV Method	Visible Method
1.	Solvent	Methanol	Methanol
2.	Absorption maxima (nm)	308	616
3.	Regression equation (Y)	Y= 0.0329x - 0.747	Y= 0.0081x + 0.0356
4.	Correlation coefficient (r^2)	0.9985	0.9989
5.	Slope (m)	0.0329	0.0081
6.	y - intercept (c)	- 0.747	0.0356
7.	Range	5 - 30µg/Ml	20 - 100µg/mL
8.	% RSD	0.61%	0.49%
9.	Limit of detection (LOD)	1.3474µg/mL	4.0577µg/Ml
10.	Limit of quantification (LOQ)	4.0833µg/mL	12.2962µg/Ml

RESULTS AND DISCUSSION

Table 1: Linearity results for UV

Concentration (µg/mL)	Absorbance
5	0.102
10	0.254
15	0.411
20	0.565
25	0.756
30	0.923

Table 2: Linearity results for Visible

Concentration (µg/mL)	Absorbance
20	0.196
40	0.352
60	0.528
80	0.692
100	0.834

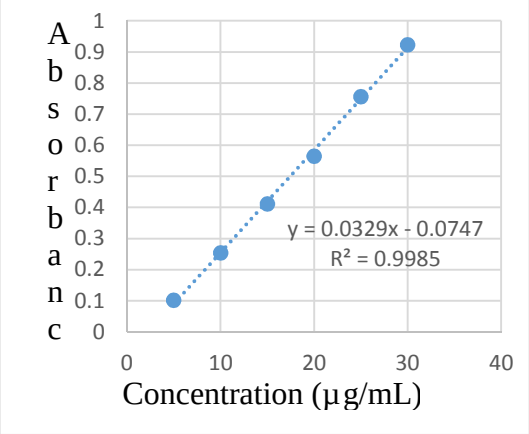

Fig 5: Linearity curve for UV

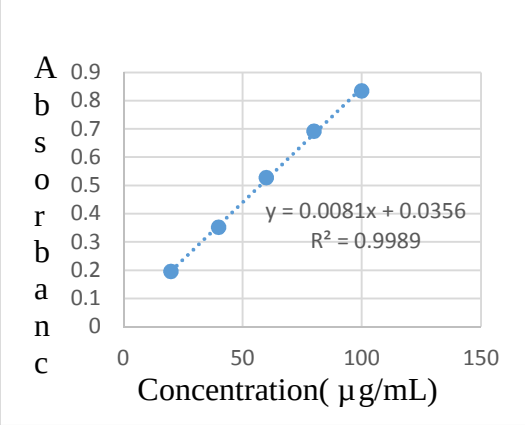

Fig 6: Linearity curve for visible.

Linearity:
The standard calibration curve was constructed between concentration and absorbance and the linearity was found in the range of 5µg/mL to 30µg/mL in UV region and 20µg/ml to 100µg/mL in visible region at their respective maxima. The regression equation and correlation coefficient were calculated and found to be within the required limits as per ICH guidelines.

Precision:
The precision of method was performed by intraday and interday variations study. The solutions of 20µg/mL in UV and 60µg/mL in visible region were prepared and their absorbance's are noted for the study.

Table 3: Precision results for UV

Concent-ration	Sample Set No	% Assay	
		Intraday	Inter day
20µg/mL	1	101.6	99.5
	2	101.2	98.4
	3	101.4	99.1
	4	101.5	99.1
	5	100.6	98.3
	6	99.3	100.5
	Mean	100.93	99.15
	SD	0.871	0.80
	% RSD	0.86	0.806

Table 4: Precision results for Visible

Concent-ration	Sample Set No	% Assay	
		Intraday	Inter day
60µg/mL	1	99.4	101.1
	2	99.6	99.2
	3	101.4	98.9
	4	101.5	99.1
	5	99.7	99.5
	6	99.3	100.2
	Mean	98.36	99.66
	SD	0.884	0.835
	% RSD	0.90	0.84

Accuracy:
The accuracy test was performed at three different concentration levels of 80%, 100% and 120% i.e. 12.0, 15.0, 18.0µg/mL solutions for UV and 32.0, 40.0, 48.0µg/mL solutions for Visible regions with three replicates at each level in which the amount of sample was kept constant i.e. 15µg in UV and 40µg in Visible regions. The percentage recovery, mean, SD and %RSD were calculated for all the 9 readings and were found to be within the limits as per ICH guidelines.

Table 5: Accuracy results for UV

S.NO	Concentration Level (%)	Amount added (µg/mL)		Amount Found (µg/mL)	% Recovery	Statistical parameters
		Std. Drug	Sample			
1	80	12	15	26.95	99.81	Mean = 99.86
2		12	15	26.98	99.92	SD = 0.055
3		12	15	26.96	99.85	% RSD = 0.06
4	100	15	15	29.91	99.70	Mean = 99.86
5		15	15	29.95	99.83	SD = 0.182
6		15	15	30.02	100.06	% RSD = 0.18
7	120	18	15	32.95	99.84	Mean = 99.85
8		18	15	32.90	99.69	SD = 0.170
9		18	15	32.01	100.03	% RSD = 0.17

Table 6: Accuracy results for Visible

S.NO	Concentration Level (%)	Amount added (µg/mL)		Amount Found (µg/mL)	% Recovery	Statistical parameters
		Std. Drug	Sample			
1	80	32	40	70.8	98.33	Mean = 98.88
2		32	40	71.5	99.30	SD = 0.499
3		32	40	71.3	99.02	% RSD = 0.50
4	100	40	40	79.9	99.87	Mean = 99.87
5		40	40	79.7	99.62	SD = 0.25
6		40	40	80.1	100.12	% RSD = 0.25
7	120	48	40	86.8	98.63	Mean = 98.36
8		48	40	85.7	97.38	SD = 0.884
9		48	40	87.2	99.09	% RSD = 0.90

Robustness: The robustness was performed by taking absorbance of six replicates of 20µg/mL in UV and 60µg/mL in Visible region by changing the wavelength by ± 1nm of selected wavelength and the results were indicated by % RSD.

Table 7: Robustness results for UV method

Concentration (µg/mL)	S. No	307nm	308nm	309nm
20µg/mL	1	0.560	0.560	0.568
	2	0.564	0.554	0.567
	3	0.561	0564	0.562
	4	0.563	0.565	0.563
	5	0.562	0.565	0.562
	6	0.560	0.564	0.564
	Mean SD	0.561 0.0016	0.562 0.00449	0.564 0.0025
	%RSD	0.29%	0.80%	0.46%

Table: 8 Robustness results for Visible method

Concentration (µg/mL)	S. No	615nm	616nm	617nm
60µg/mL	1	0.521	0.528	0.526
	2	0.517	0.526	0.521
	3	0.521	0522	0.526
	4	0.525	0.529	0.522
	5	0.524	0.522	0.524
	6	0.521	0.527	0.523
	Mean SD	0.523 0.002	0.525 0.003	0.523 0.002
	%RSD	0.49%	0.57%	0.39%

Limit of Detection and Limit of Quantification:

For UV

The Limit of detection was found to be 1.3474µg/mL

The Limit of quantification was found to be 4.0833µg/mL

For Visible

The Limit of detection was found to be 4.0577µg/mL

The Limit of quantification was found to be12.2962µg/mL

CONCLUSION

The proposed methods were rapid, accurate, precise and sensitive for the quantification of Capecitabine from its pharmaceutical dosage forms by the multivariate spectrophotometric method. The methods rely on the use of simple working procedure, and hence this method can be routinely employed in quality control for analysis of Capecitabine in bulk drug and formulation.

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