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Development and Validation of New Analytical Methods for the Estimation of Capecitabine in Pharmaceutical Dosage Form

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ABSTRACT

A validated method for the determination of Capecitabine has been developed by using reverse phase high performance liquid chromatography and UV spectrophotometry in pharmaceutical dosage forms. Spectrophotometric determination was carried out at an absorption maximum of 307 nm using Ethanol. The linearity over the concentration range of 5-25 µg/ml with correlation coefficient 0.999 are obtained. Chromatographic separation was carried out using a mobile phase of Acetonitrile and 0.015M Phosphate buffer(pH adjusted to 3.2 with Ortho phosphoric acid) in the ratio of 55:45 (V/V) on Agilent C18 column (250 X 4.6 mm, 5µm) in an isocratic mode at a flow rate of 1.1 ml/min. with detection at 306 nm using a UV detector. The developed methods were found to be precise and accurate for the estimation of Capecitabine in pharmaceutical dosage forms.

Keywords: Capecitabine, RP-HPLC, Spectrophotometry, UV detector.

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INTRODUCTION

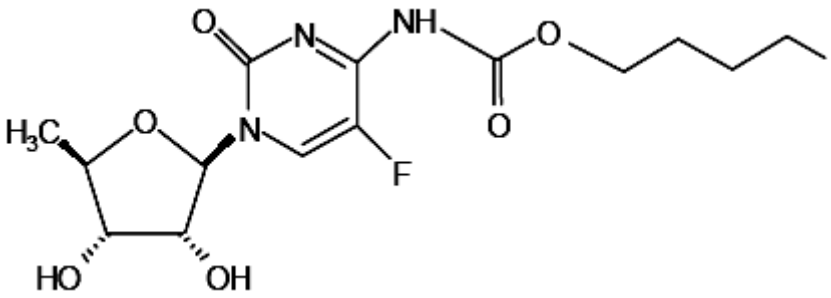

Fig 1: Chemical structure of Capecitabine

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity and it is in a class drugs known as anti metabolites. The chemical structure of Capecitabine was shown in **Fig.1**. Capecitabine is an orallyadministered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [1,2]. Capecitabine 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. 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 with empirical formula of C15H22FN3O6 and the molecular weight of 359.35 g/mol [3,4]. It elicits the pharmacodynamic response by resembling as a normal cell nutrient needed by cancer cells to grow. The cancer cells take up the Capecitabine, which then interferes with their growth. The length of treatment depends on the types of drugs you are taking, how well your body responds to them, and the type of cancer you have. Literature review reveals that few analytical methods have been evoked for the estimation of Capecitabine by spectrophotometric [5] and HPLC [6-11] methods were reported. In present study the authors were developed a sensitive, accurate and reliable method for the estimation of Capecitabine in bulk and pharmaceutical dosage forms.

Experimental:

a) Instrumentation:

The method involved in estimation of the Capecitabine uses HPLC Agilent(1120) Module with UV Detector Chromatograph and Agilent, C_{18} 250× 4.6 mm. 5µ. as column and for UV method uses Shimadzu UV-1800 spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software. In the present work the method devolpment and validation of the Capecitabine is carried out by using the RP-HPLC chromatographic method and UV spectrophotometric method.

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b) Chemicals and Reagents:

HPLC grade acetonitrile and water, AR grade Phosphate buffer (0.05 M), Orthophosphoric acid, HPLC grade Methanol, ethanol.

c) Chromatographic conditions:

HPLC chromatographic separation was carried out in an isocratic mode utilizing Agilent C18 column with dimensions (5µ, 250mm x 4.6mm) as stationary phase with injection volume of 20µl. The mobile phase composed of Acetonitrile and 0.015M Phosphate buffer (pH adjusted to 3.2 with Ortho phosphoric acid) in the ratio of 55:45 at a flow rate of 1.1 ml/min. with UV-detection at 306 nm at 3.433min shown in Fig.2.

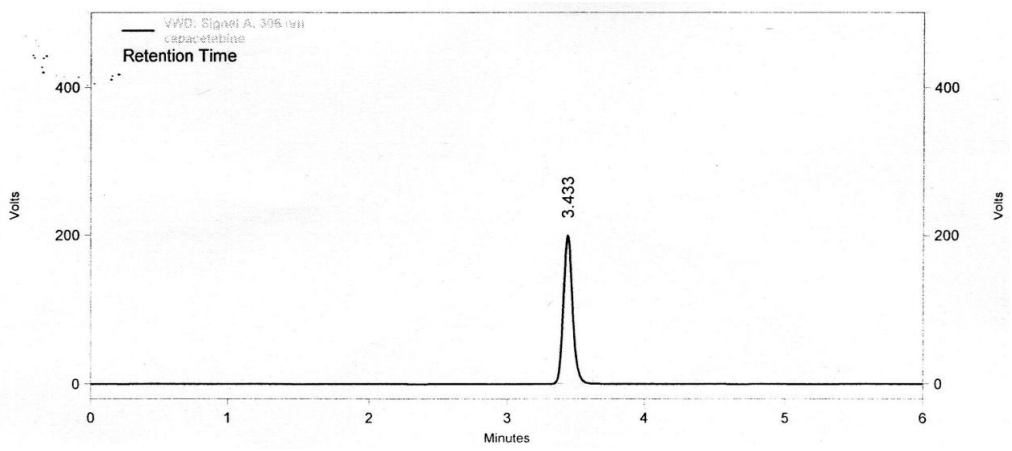

Fig 2: Chromatogram showing Retention Time (R_t) at 3.43333min for capecitabine.

d) Spectrophotometric conditions:

In this method Capecitabine was dissolved in Ethanol which showed the absorption maximum at 307 nm.

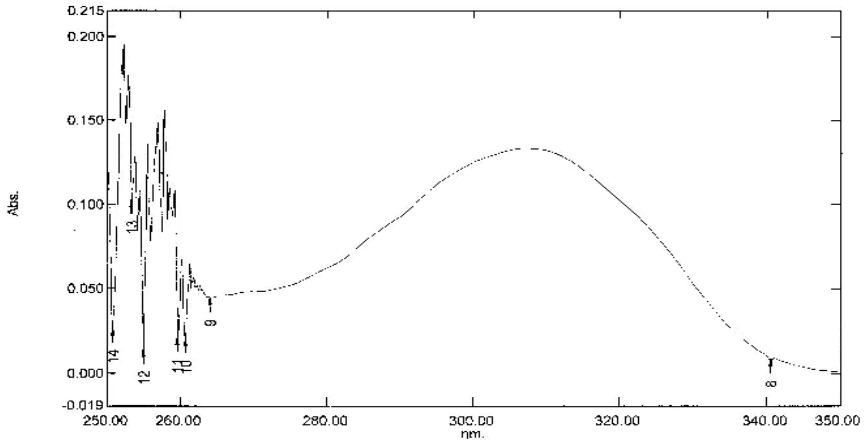

Fig 3: Zero order spectra for capecitabine at 307nm:

ISSN: 0975-8585

Preparation of standard solutions:

Spectrophotometry :

About 100mg of Capecitabine was accurately weighed and transferred into a 100 ml volumetric flask and diluted to volume with ethanol to get the stock solution (1mg /ml). From this stock solution, pipette out 10ml, placed in to 100ml volumetric flask and volume was made up to mark with water to give a solution containing 100µg/ml. From this five dilutions were prepared in between 5-25 µg mL^{-1} of Capecitabine with ethanol was used in spectrophotometric estimation,

RP-HPLC:

Accurately weighed 100 mg of Capecitabine in 100 mL volumetric flask and dissolved in ethanol and the volume were made up to the mark with the same solvent. From the above 10 mL solution were pipette out into separate 100 mL volumetric flask and volume was made up to the mark with the same solvent. This gave the concentration of 100 µg mL^{-1} of Capecitabine, from this five dilutions were prepared. Five dilutions in between 5-25 µg mL^{-1} of Capecitabine with ethanol was used in spectrophotometric estimation, but in case of HPLC the final working concentration make up by using mobile phase as a solvent.

Sample preparation:

Spectrophotometry:

Twenty tablets were weighed and finely powdered. The powder equivalent to 100mg of Capecitabine was accurately weighed and transferred to volumetric flask of 100ml capacity containing 25ml of the ethanol and sonicated for 5 min. The flask was shaken and volume was made upto the mark with ethanol to give a solution of 1000µg/ml. The above solution was filtered through whatmann filter paper(No.41). From this solution 10ml was diluted to 100ml with ethanol to give a solution of 100µg/ml and used for the estimation of Capecitabine.

RP-HPLC:

Twenty tablets each containing 150 mg of Capecitabine were weighed and powdered for further study. The powder equivalent to 150 mg of Capecitbaine was accurately weighed and transferred to 100 mL volumetric flask containing 50 mL of the ethanol and sonicated for 10 min. The above solution was carefully filtered through Whatmann filter paper (No. 41) and the residue was washed with 3 portions of 10 mL of solvent. The volume was made up to the mark with ethanol. From this solution, required dilutions for UV method were prepared within the linearity range using ethanol as solvent, but in case of HPLC, the final working concentration prepared by using mobile phase as a solvent.

From this, suitable dilutions were made to obtain the concentrations ranging from 80, 100, 120 µg/ml.

Procedure:

Spectrophotometry :

Five aliquots of Capecitabine aqueous solution were and transferred to respective 10 ml volumetric flasks in such amounts as to obtain final concentrations of 5, 10, 15, 20 and 25 µg/ml of Capecitabine . Volume was completed with Ethanol and each flask content was measured to determine 307 value.

RP-HPLC:

Various standard concentrations of Capecitabine ranging from 30-80 µg/ml were prepared in mobile phase. The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed with a sonicator (EQUITRON-230VAC, 50Hz) for 15 min. And pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug, the mobile phase was pumped for about 30 min. To saturate the column there by to get the base line corrected, then 20 µl of each of the drug solution was injected for five times. Quantitative determinations were made by comparison of the peak area from a standard injection. The amount of Capecitabine present in the sample was calculated through the calibration curve.

RESULTS

Linearity:

Calibration curve for spectrophotometric method was constructed by plotting absorbance Vs concentration of solution. For chromatographic method it was constructed by plotting peak area against concentration of solution. Figs. 4 and 5 show spectrophotometric and HPLC linearity curves of Capecitabine. Linearity ranges and correlation coefficients obtained from these methods are presented in Table 1.

Table 1: Analytical parameters of Capecitabine

S.NO	Parameter	Spectrophotometry	RP-HPLC	Acceptance Criteria
1	Linearity range (µg /ml)	5-25	30-80	----------
2	Slope (m)	0.038	37353	----------
3	Intercept (c)	0.007	43473	----------
4	Correlation coefficient (r)	0.999	0.999	NLT 0.995
5	Precision (Repeatability) %RSD	0.90%	0.59%	NMT 2%
6	Precision (Intermediate) %RSD	0.91%	0.58%	NMT 2%
7	Accuracy	99.9%	101%	98-102%
8	Limit of detection (µg mL^{-1})	1.15	1.15	----------
9	Limit of Quantitation (µg mL^{-1})	3.48	19.776	----------

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Table 2: System suitability parameters

S.NO	Parameters	RP-HPLC
1	Retention time (min.)	3.433
2	Theoretical plates	12618
3	Tailing factor	1.15

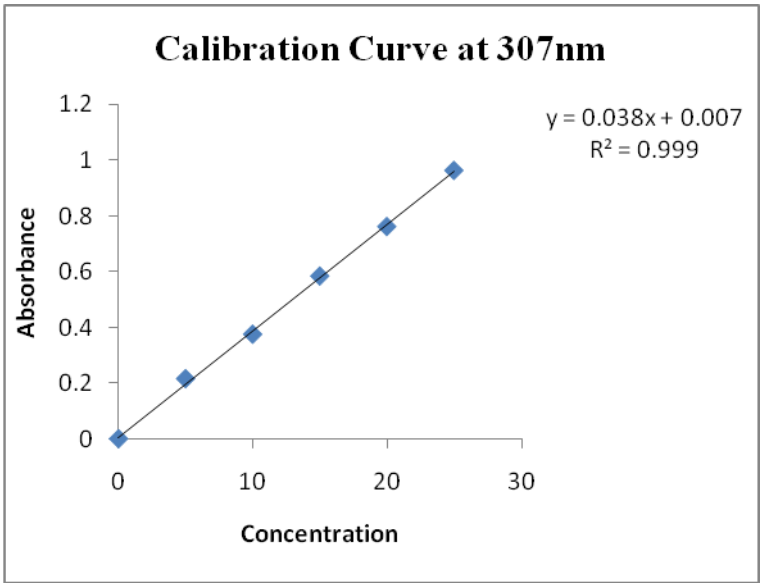

Fig 4: Linearity Curve of Capecitabine(Spectrophotometry)

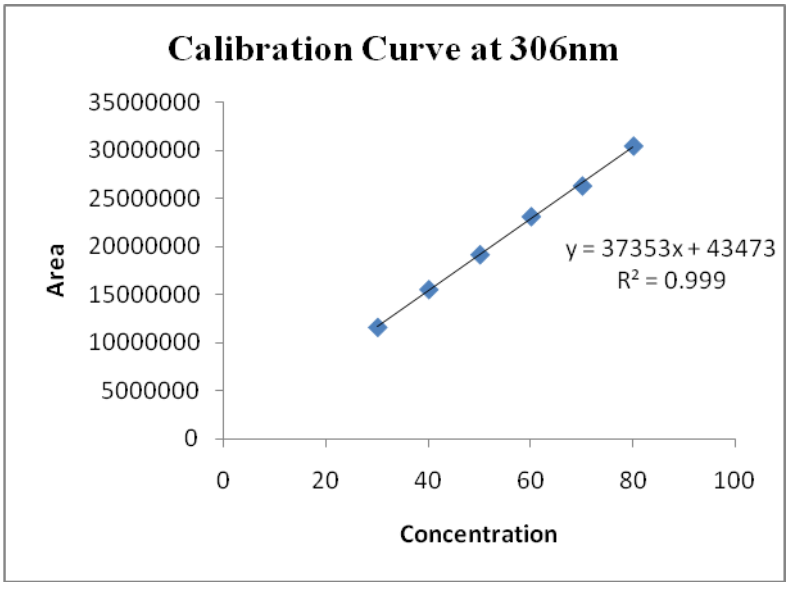

Fig 5: Linearity Curve of Capecitabine(RP-HPLC)

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System suitability parameters:

The system suitability tests were carried out on freshly prepared standard stock solution of Capecitabine under the optimized chromatographic conditions. The parameters that were studied to evaluate the suitability of the system were: a) No. of theoretical plates b) tailing factor c) retention time. These values are presented in Table 2.

Precision for RP-HPLC:

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. It was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for the title ingredient. The repeatability (within-day in triplicates) and intermediate precision (for 3 days) were carried out at six concentration levels for compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for Capecitabine was found to be 0.5998 for intra-day studies. The values for inter-day studies was 0.5898. This shows that values are not more than 2%, indicates that the developed method is precise. The precision expressed as % RSD is given in Table 3. and Table 4.

Table 3: Precision (Repeatability) study results by RP-HPLC

Precision	Conc(µg/ml)	Area±SD	%RSD
CAPECITABINE	30	11215495±459672.3	0.8472
	40	15271664±456855.1	0.9823
	50	18864957±570870.5	0.5789
	60	22739054±537857.7	0.4052
	70	26101385±771722.3	0.3886
	80	29874089±735092.3	0.3966

a Repeatability, 2 experiments three replicates of six concentration levels within-day

Table 4: Precision (Intermediate Precision) study results by RP-HPLC

Precision	Conc(µg/ml)	Area±SD	%RSD
CAPECITABINE	30	10808825±75374.16	0.6973
	40	14875649±108112.6	0.7267
	50	18254132±122699.9	0.6721
	60	22217468±113744.9	0.5119
	70	25334856±135194.9	0.5336
	80	29202936±116028.9	0.3973

a Intermediate precision,2 experiments three replicates of six concentration levels between-days (2-days)

Precision for UV:

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Sample – to sample precision were evaluated using, three samples of

three different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The value of %RSD for Capecitabine was found to be 0.91842 for intra-day studies. The values for inter-day studies was 1.07. This shows that values are not more than 2%, indicates that the developed method is precise. The precision expressed as % RSD is given in Table 5.

Table 5: Determination of precision results for Capecitabine at 307nm by zero derivative spectroscopy

Conc (µg/ml)	Inter-day Absorbance ± SD*	%RSD	Intra-day(Repetability) Absorbance ± SD*	%RSD
5	0.140875 ± 0.001727	1.22583	0.143125 ± 0.001356	0.947565
10	0.27425 ±0.004464	1.627764	0.285625 ± 0.002875	1.0067
15	0.40625 ± 0.001982	0.487892	0.408875 ± 0.0029	0.709293
20	0.548875 ± 0.007492	1.364912	0.559125 ± 0.005194	0.929029
25	0.686875 ± 0.004422	0.643777	0.69675 ±0.006964	0.999526

* Average of Eight determination

Assay and recovery study:

To determine the accuracy of the proposed methods, recovery experiments were carried out by standard addition method. The values of recovery experiments and assay of commercial formulations are presented in Table 6.

Table 6: Assay of commercial formulations by proposed methods

S.NO	Labelled amount (mg)	Spectrophotometry			RP-HPLC		
		Amount added	Amount Recovered	% Recovery*	Amount added	Amount Recovered	% Recovery*
1	150	120	149.4	99.6	120	150.15	100.1
2	150	150	150.75	100.5	150	153	102
3	150	180	149.55	99.7	180	152	101

*Mean of three determinations

DISCUSSION

The linearity was obeyed in the range of 5-25 µg/ml for spectrophotometric method and 30 – 80µg/ml for chromatographic method. Quantitative estimation of formulations showed the %Recovery for Spectrophotometry is 99.6 to 100.5. For Chromatographic method the %Recovery is 99.1 to 100.2. System suitability indicates that the developed method has acceptable accuracy and precision.

ISSN: 0975-8585

CONCLUSION

The developed method is an alternative to determine Capecitabine in pharmaceutical dosage forms that contain it as unique active principle with quite satisfactory results for the specific purposes of its design. Its advantages over other existing methods are its simplicity, fastness, low-cost and non-polluting conditions.The developed methods are simple, accurate and reproducible, so these methods are suitable to determine Capecitabine in formulations.

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