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DEVELOPMENT AND VALIDATION OF CAPECITABINE TABLET (PHARMACEUTICAL DOSAGE FORM) BY USING RP-HPLC METHOD.

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Abstract:

A new precise accurate and reliable validated method for the determination of Capecitabine was developed by using reverse phase high performance liquid chromatography in pharmaceutical dosage forms. Spectrophotometer determination was carried out at an absorption maximum of 240nm by using methanol. The linearity was over the concentration range of 20-120 µg/ml with correlation coefficient 0.999. Chromatographic separation was carried out by using a mobile phase of methanol: Acetonitrile: water (80:20:80 V/V) on Waters 2487 dual absorbance column in an isocratic mode at a flow rate of 1.1 ml/min with UV detection at 240 nm. The developed methods were found to be precise and accurate for the estimation of Capecitabine in pharmaceutical dosage forms and could be used for routine analysis.

Keywords: *Capecitabine, RP-HPLC, Spectrophotometry, Waters 2487 dual absorbance detector, Nova pack 300 × 3.9mm 5µ as column, 240nm*

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INTRODUCTION:

Capecitabine is a fluoropyrimidine carbonate with antineoplastic activity and it is in a class drugs known as anti-metabolites. Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] – cytidine. Capecitabine is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR), which is enzymatically converted to 5-fluorouracil in the tumour cells, where it inhibits DNA synthesis and slows growth of tumour tissue. The activation of Capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5- fluoro cytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR), to form 5-fluorouracil. Capecitabine reached peak blood levels in about 1.5hours (T_{max}) with peak 5-FU levels occuring slightly later, at 2hours. Food reduce both the rate and extent of absorption of Capecitabine with mean C_{max} and $AUC_{0-\alpha}$ decreased by 60% and 35%, respectively. The Cmax and $AUC_{0-\alpha}$ of 5-FU were also reduced by food by 43%and 21% respectively. Food delayed T_{max} of both parent and 5-Fuby 1.5hours.Plasma protein binding of Capecitabine and its metabolites is less than 60% and is not concentration-dependent. Capecitabine was primarily bound to human albumin (approximately 35%) Capecitabine is extensively metabolised enzymaetically to 5-FU.the enzyme dihydropyrimidine dehydrogenase hydrogenates 5-FU, the product of Capecitabine metabolism, to the much less toxic 5-fluoro 5, 6 dihydro-fluorouracil (FUH_2) dihydropyrimidinase claves the pyrimidine ring to yield 5-fluorto-ureido propionicacid (FUPA). Finally, β-ureido-pripionase cleaves FUPA to α-fluoro-β-alanine (FBAL) which is cleared in the urine.

MATERIALS AND METHODS:

Chemicals and Reagents: HPLC grade acetonitrile, methanol.0.1N NaOH and water were purchased from Hetero Labs Ltd, Jadcherla, and Hyderabad, India.**Preparation of standard solutions:** About 100 mg of Capecitabine was accurately weighed and transferred into a 100 ml volumetric flask and diluted to volume with methanol to get the stock solution. This gave a concentration of 1000 µg / ml.

Preparation of working stock solutions: 0.6 ml of stock solution was pipetted out and placed in a 10 ml volumetric flask and the volume was made up to mark with methanol. This gave a solution containing 60µg/ml.

Preparation of mobile phase: A mixture of Methanol, acetonitrile and water (80:18:2 v/v) was employed as a mobile phase. 400 ml of methanol, 90 ml of ACN and 10 ml of water was mixed and sonicated for 15 min. This was filtered by using a 0.45 µm filter paper.

Sample preparation: Ten tablets were weighed and finely powdered. The powder equivalent to 100 mg of Capecitabine accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the methanol and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. The above solution was filtered through 0.45 µm filter paper. From this solution 0.6 ml was diluted to 10 ml with methanol to give a solution 60 µg/ml.

Determination of λmax by UV spectrophotometer: The standard solutions of Capecitabine were scanned in the range of 200-400nm against methanol as a blank. Capecitabine showed maximum absorbance at 240 nm. So the wavelength selected for the determination of Capecitabine was 240 nm.

RESULTS AND DISCUSSION:

Method development: HPLC chromatographic separation were carried out in an isocratic mode by using the following optimized conditions and the corresponding chromatogram.

Chromatographic conditions:

Mobile phase: Filter and degassed mixer of water and acetonitrile in the ratio of 80 : 20
Column: Nova pack 300x3.9 mm 5 µ
Flow rate: 1.0 ml /minute
Wavelength: 240 nm
Load: 10 µl
Run time: 60 min
Column temperature: 35 °C

Sample preparation: Weigh 25 mg of Capecitabine WS in 50ml volumetric flask dissolve and dilute to 50ml with mobile phase. The retention time Capecitabine is about 20.0 min. In this trial all impurities are separated from the main peak. The specificity test of the proposed method was demonstrated by blank interference i.e. the blank chromatogram did not interfered with that of the drug peak.

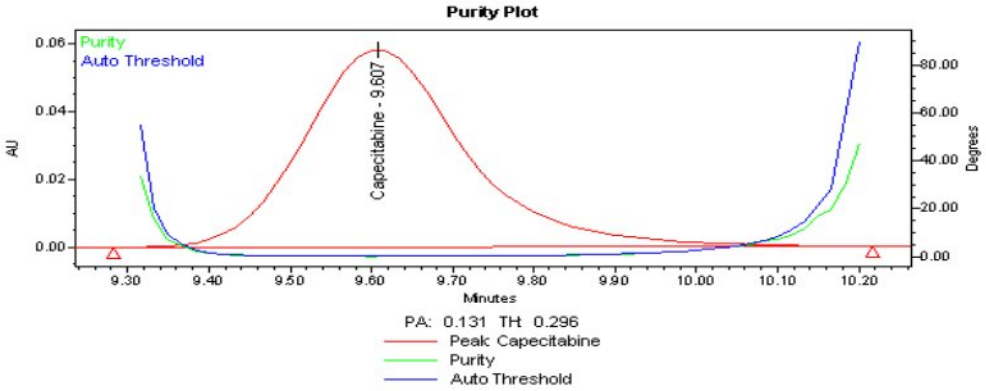

Fig 1:Purity plot of Acid stressed Capecitabine tablets

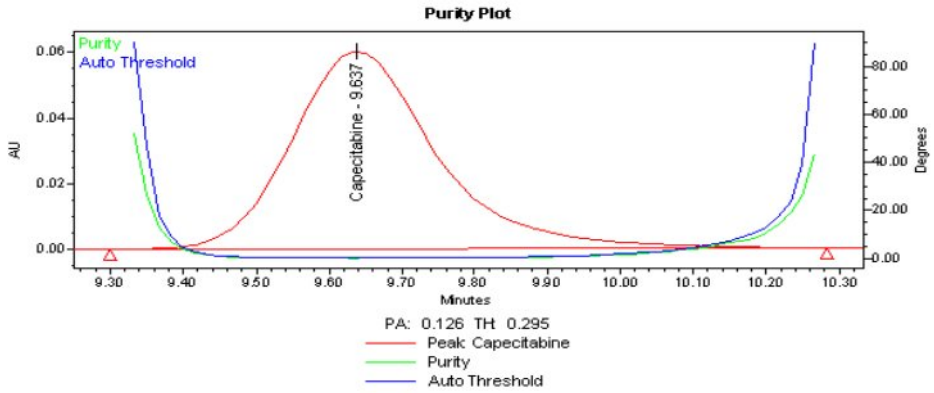

Fig 2: Purity plot of Thermal heat stressed Capecitabine tablets

Linearity and range: Chromatographic method was tested for linearity by plotting peak area against concentration of solution. Linearity ranges and correlation coefficients obtained were presented in Table 1.

TABLE 1: LINEARITY OF TEST METHOD

Spike level	Average Amount added (in mg/ml)	Average Amount recovered (in mg/ml)
25%	0.0260	0.0265
50%	0.0502	0.0509
100%	0.1014	0.1034
150%	0.1544	0.1588
200%	0.1953	0.1965

Linearity of Test Method

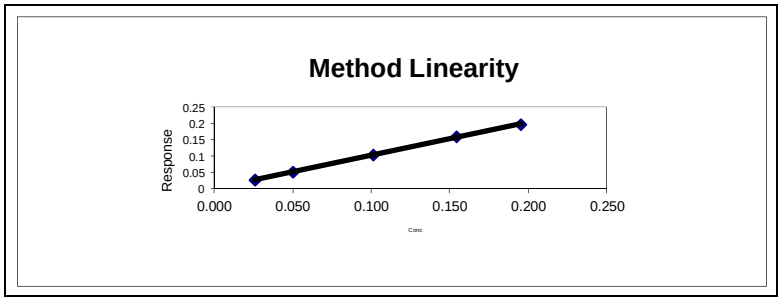

Fig 3: Linearity of Test Method

Accuracy of the method:

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting triplicate injections at three different concentrations levels equivalent to 25%,50 %, 100 %,150 % and 200% of the active ingredient, by adding a known amount of Capecitabine standard to a sample of known concentration and calculating the recovery of Capecitabine with % RSD and % recovery for each concentration. The mean %recoveries were in between 98.22-101.49% and were given in table2.

TABLE 2: Accuracy of Capecitabine

Sample No.	Spike level	'mg/ml' added	'mg/ml' found	Mean % Recovery	
1.	25%	0.0258	0.0265	102.8	101.7
2.	25%	0.0257	0.0262	101.9	
3.	25%	0.0266	0.0267	100.4	
4.	50%	0.0504	0.0514	101.9	101.3
5.	50%	0.0495	0.0500	101.0	
6.	50%	0.0507	0.0512	100.9	
7.	100%	0.1025	0.1053	102.7	101.9
8.	100%	0.1011	0.1027	101.6	
9.	100%	0.1007	0.1022	101.5	
10.	150%	0.1519	0.1564	102.9	102.8
11.	150%	0.1549	0.1592	102.8	
12.	150%	0.1568	0.1608	10.28	
13.	200%	0.1950	0.1963	100.6	100.6
14.	200%	0.1975	0.1998	101.2	
15.	200%	0.1933	0.1934	100.1	

The Mean recovery of Capecitabine at each spike level should be not less than 97.0% and not more than 103.0%. The Mean recovery of Capecitabine at each spike level is within the specification.

Precision of the method:

The intra-day and inter-day variations of the method were determined by using six replicate injections of one concentration and analysed on the same day and three different days over a period of two weeks. The result revealed that, the precision with %RSD (0.66% and 1.02%) respectively for intra-day and inter-day. Results of intra-day and inter-day precision studies are shown in table3.

The relative standard deviation for six % assay results should be not more than 2.0% for both the Analysts. The Assay of Capecitabine should be not less than 97.0% and not more than 103.0%. The observed assay results of Capecitabine are within in the specifications.

Limit of detection (LOD) and Limit of quantification:

To determine the Limit of Detection the sample was dissolved by using Mobile Phase and injected until peak was disappeared. After 2µg/ml dilution, Peak was not clearly observed. So it confirms that 0.625 µg/ml is limit of Detection. And Limit of Quantification found to be 2µg/ml. For this study six replicates of the sample at lowest concentration were Measured and quantified. The LOD and LOQ were found to be 0.6µg/ml and 2µg/ml respectively.

TABLE 3: Precision of Capecitabine

System suitability Parameters	Observed value		Acceptance Criteria
	Analyst – 1	Analyst- 2	
Tailing factor of Capecitabine	1.52	1.51	NMT 2.0
Relative standard deviation of Capecitabine area from five replicate injections of standard	0.49	0.11	NMT 2.0%

INTERMEDIATE PRECISION (ANALYST TO ANALYST VARIATION)

TABLE 4: INTERMEDIATE PRECISION

Sample No.	Assay of Capecitabine as % of labeled amount	
	Analyst – 1	Analyst – 2
1	99.0	99.3
2	98.3	98.7
3	99.2	98.5
4	98.5	99.1
5	98.8	98.4
6	98.3	98.1
Mean	98.68	98.7
% RSD	0.4	0.5

Robustness:
The robustness study was performed by slight modification in flow rate and composition of the mobile phase. Capecitabine at 60µg/ml concentration was analyzed under these changed experimental conditions. In this study, the chromatographic parameters monitored were, retention time, area, capacity factor, tailing factor and theoretical plates. The obtained results of robustness study are shown in table5.

The difference of % assay result from centrifuged sample to filtered samples should be not more than 3.0 the differences of % assay results from centrifuged sample to filtered samples are within the specification

Ruggedness:
Inter day variations were performed by using six replicate injections of standard solution of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

TABLE 5: System Suitability Parameters (robustness study)

System Suitability Parameters	Observed value			Acceptance Criteria
	90% of method organic phase	100% of method Organic phase	110% of method Organic phase	
Tailing factor	1.48	1.52	1.54	NMT 2.0
RSD of five injections of standard	0.06	0.10	0.06	NMT 2.0%

System Suitability Parameters	Observed value with Flow rate			Acceptance Criteria
	0.8 mL/min	1.0 mL/min	1.2 mL/min	
Tailing factor	1.61	1.51	1.45	NMT 2.0
RSD five injections of standard	0.52	1.20	0.26	NMT 2.0%

Centrifuged		Sample filtered through PVDF filter				Sample filtered through Nylon 66 filter			
% Assay		% Assay		Difference		% Assay		Difference	
98.8	98.9	100.7	98.5	1.9	0.4	98.5	96.9	0.3	2.0

TABLE 6: System Suitability Parameters (Ruggedness)

System suitability Parameters	Observed value		Acceptance Criteria
	System – 1	System – 2	
Tailing factor of Capecitabine peak	1.52	1.36	NMT 2.0
Relative standard deviation of Capecitabine peak area from five replicate injections of standard	0.49	0.3	NMT 2.0%

TABLE 7: Assay of Capecitabine

Sample No.	Assay of Capecitabine as % of labeled amount	
	System – 1	System – 2
1	99.0	98.7
2	98.3	98.8
3	99.2	98.0
4	98.5	98.4
5	98.8	98.6
6	98.3	99.0
Mean	98.68	98.58
RSD	0.4	0.4

System suitability parameters:
The system suitability tests were carried out on freshly prepared standard solution (60µg/ml) of Capecitabine under the optimized chromatographic conditions .from that the parameters that were studied to evaluate the suitability of the system were: a) No. of theoretical plates b) tailing factor c) retention time.

CONCLUSION:

In this thesis the author made a humble attempt in developing "New RP-HPLC methods for estimation of assay of Capecitabine tablet and its known impurities" with the facilities, and the results are incorporated in this thesis. Opens with the general introduction, aim and plan of work introduction to drug and its known impurities used in the present study, Introduction to HPLC, General guidelines for HPLC method development and guidelines for method validation. The results indicate that the proposed methods are sensitive, accurate, precise, simple and reproducible and can be used for routine determination of Capecitabine tablets and its impurities in bulk drug samples and pharmaceutical formulations.

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