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METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF CAPECITABINE BY RP-HPLC METHOD

Azka fathima *and Shaik Gazi

Department of pharmaceutical Analysis &QA, Deccan school of pharmacy,Hyderabad -500001

Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Capecitabine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6×150mm, 5µm) column using a mixture of Acetonitrile: Water (50:50v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 245 nm. The retention time of the Capecitabine was 2.4 ±0.02min. The method produce linear responses in the concentration range of 15-75mg/ml of Capecitabine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Capecitabine, RP-HPLC, validation.*

Corresponding Author:

Azka fathima,

Department of Pharmaceutical Analysis &QA,
Deccan School of Pharmacy,
Hyderabad -500001

QR code

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP) Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs are introduced into the market. Also quality is important in every product or service, So, it becomes necessary to develop new analytical methods for such drugs (Dr. Kealey and P.J Haines.

Capecitabine (CAP)151 is a prodrug which is enzymatically changed to 5- fluorouracil in the tumor and stops DNA synthesis there by reduces growth of the tumor tissue. It is an orally administered chemotherapeutic drug which has a very good potency in the treatment of various kinds of cancer diseases especially in the therapy of colorectal cancer, breast cancer, gastric cancer and esophageal cancer.

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.

MATERIALS AND METHODS:

Materials

Capecitabine was received as a gift sample from cipla pvt.Ltd. (Goa) .water and methanol for HPLC ,acetonitrile for HPLC were procured from S.d fine chemicals, Mumbai,india

SELECTION OF WAVELENGTH

By using UV spectrophotometer the maximum wavelength absorbance was noted at 245 nm which is taken as basis to detect wave length of CAP.

Preparation of mobile phase:

Accurately measured 500ml (50%) of HPLC Acetonitrile and 500ml of Water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of Assay Standard Solution:

Accurately weighed and transferred 10mg of Capecitabine working standard into a 10ml of clean dry volumetric flasks, added about 7ml of Diluents and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.45ml of the above Capecitabine stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation of Sample Solution:

Calculate average weight of the tablet and then accurately weighed and transferred tablet powder equivalent to 10mg of Capecitabine into a 10ml clean dry volumetric flask and added about 10mL of diluent and it was sonicated to dissolve completely and made volume up to the mark with same solvent.

Further pipette 0.45ml of the above Capecitabine stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents.

Procedure:

Injected the three replicate injections of standard and sample solutions and calculated the assay by using formula:

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{100} \times \frac{\text{Purity}}{\text{Label claim}} \times \frac{\text{Avg weight of tablet}}{\text{Weight of sample}} \times 100$$

Analytical Method Validation:

VALIDATION PARAMETERS

- Accuracy
- Precision
- Linearity
- Limit of Detection
- Limit of Quantitation
- Robustness
- System Suitability
- **Accuracy:**
- **Procedure:**
- Injected the Three replicate injections of individual concentrations (50%,100%,150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculated the Amount found and Amount added for Capecitabine and calculated the individual recovery and mean recovery values.
- **Acceptance Criteria:**
- The % Recovery for each level should be between 98 to 102 %
- The % Recovery for each level should be between 98 to 102 %
- **Precision:**
- **Preparation of Capecitabine Solution For Precision:**
- Accurately weighed and transferred 10mg of Capecitabine working standard into a 10ml of clean dry volumetric flasks, added about 7ml of Diluents and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)
- Further pipette 0.45ml of the above Capecitabine stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents.
- The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.
- **Acceptance Criteria:**
- The % RSD for five standard injection results should not be more than

- **Linearity:**

Injected each level into the chromatographic system and measured the peak area.

Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient.

- **Acceptance criteria:** Correlation coefficient should be not less than 0.99
- **Limit of Detection:**
- The detection limit is determined by the analysis of sample with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.
- **Limit of Quantitation:**
- The quantitation limit is generally determined by the analysis of sample with known concentration of analyte and by establishing that minimum level at which the analyte can be quantified with acceptable accuracy and precision.
- **Robustness:**
- The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

RESULTS AND DISCUSSION:

- **Optimized Chromatogram (Standard)**

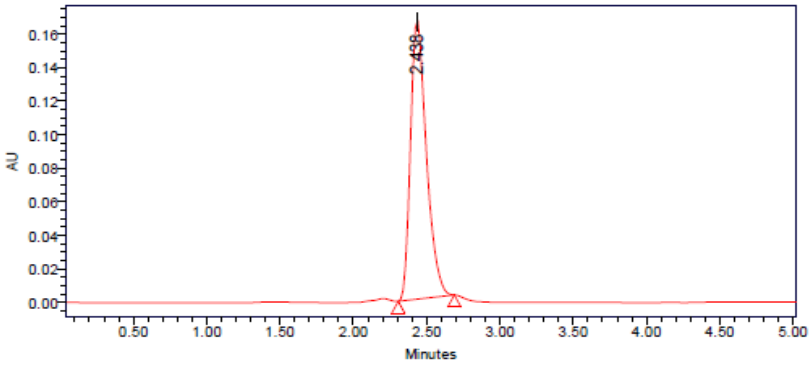


Figure1: Optimized Chromatogram (Standard)

Table1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Capecitabine	2.438	1192631	63821	1.1	7295

VALIDATION

Blank:

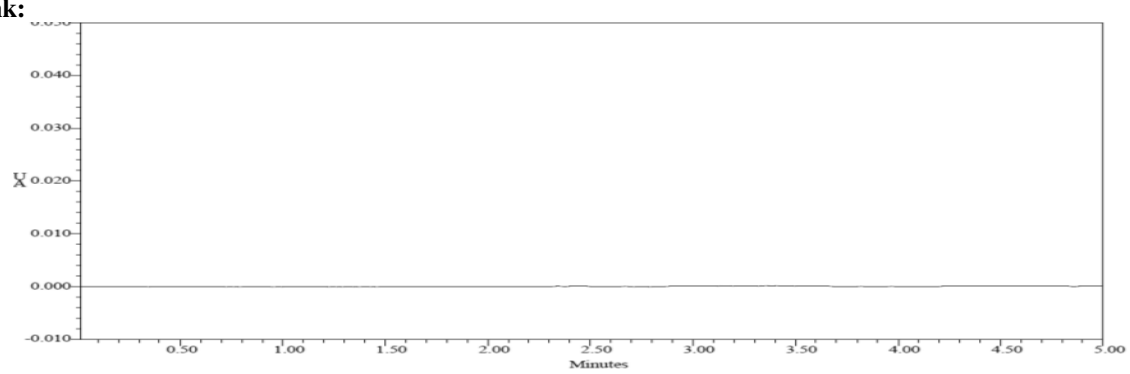


Fig2: Chromatogram showing blank (mobile phase preparation)

System suitability:

Fig: Chromatogram showing injection -5

Table: Results of system suitability for Capecitabine

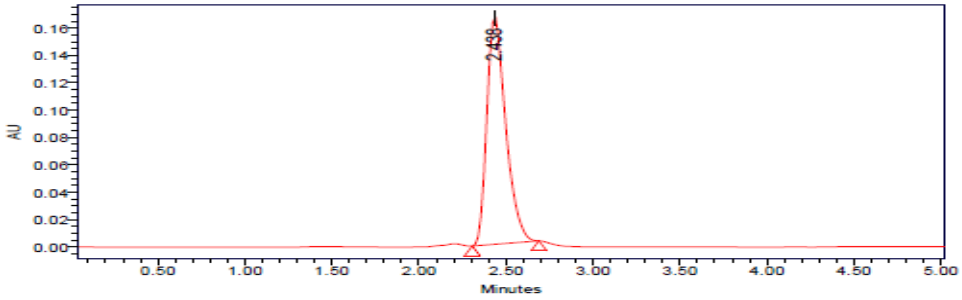


Fig3: Chromatogram showing injection -5

Table2: Results of system suitability for Capecitabine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP plate count
1	Capecitabine	2.438	1255499	165949	1.35	7348
2	Capecitabine	2.438	1255377	166036	1.34	7350
3	Capecitabine	2.438	1256231	165935	1.35	7342
4	Capecitabine	2.435	1256794	166852	1.37	7360
5	Capecitabine	2.436	1252808	167387	1.36	7416
Mean			1255342			
Std. Dev.			1528.533			
% RSD			0.121762			

- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Capecitabine in drug product.

Assay (Standard) :

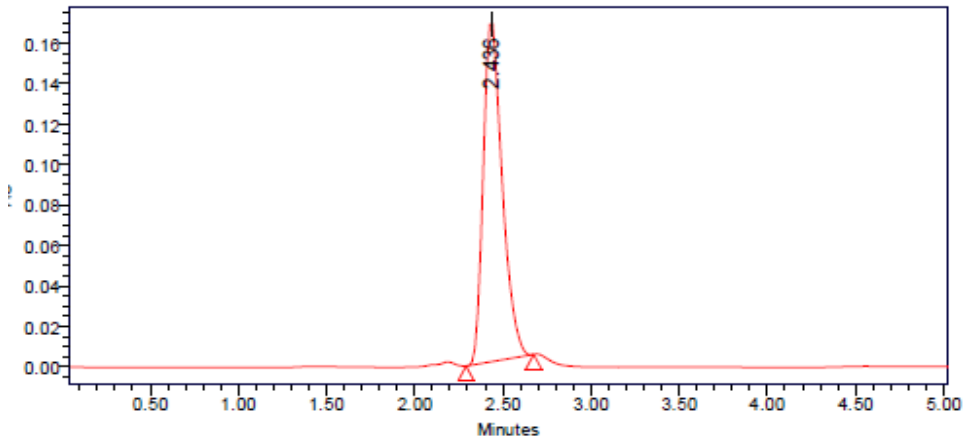


Fig4: Chromatogram showing assay of standard injection -1

Table3: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Capecitabine	2.436	1231073	167575	1.32	5408
2	Capecitabine	2.436	1228284	169301	1.31	6428
3	Capecitabine	2.437	1222835	167010	1.30	4332

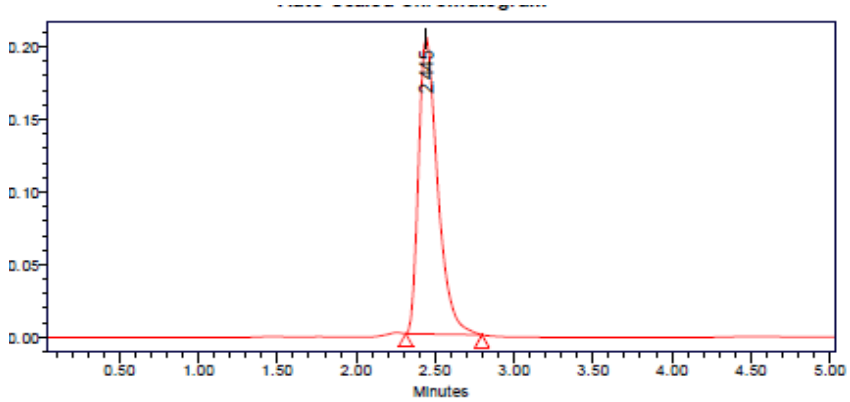
Table4: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Capecitabine	2.436	1237777	167575	1.32	7408
2	Capecitabine	2.436	1228292	169301	1.31	4428
3	Capecitabine	2.437	1220510	167010	1.30	6332

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

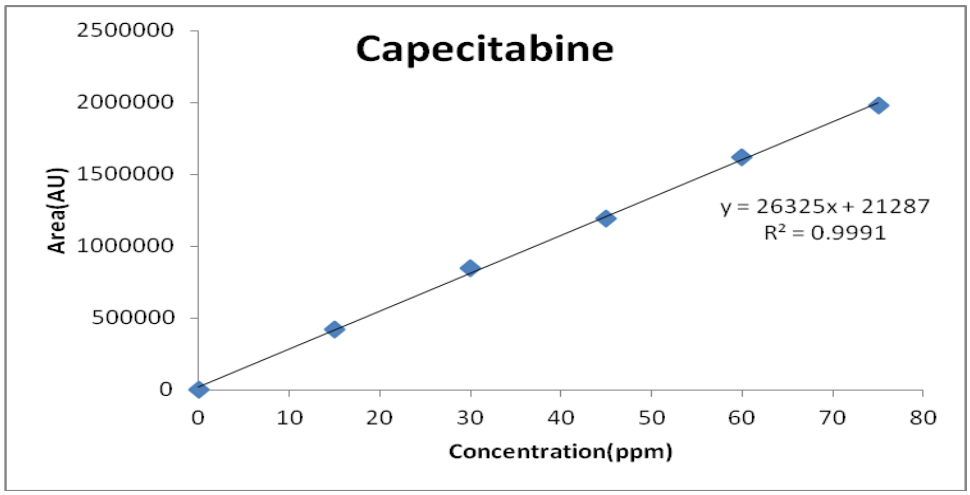
The % purity of Capecitabine in pharmaceutical dosage form was found to be 99.9%.

LINEARITY



CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	15	421796
80	30	842946
100	45	1191428
120	60	1618010
140	75	1976727



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Capecitabine is a straight line.

Y = mx + c

Slope (m) =26325

Intercept (c) = 21287

Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 21287. These values meet the validation criteria.

Precisi REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

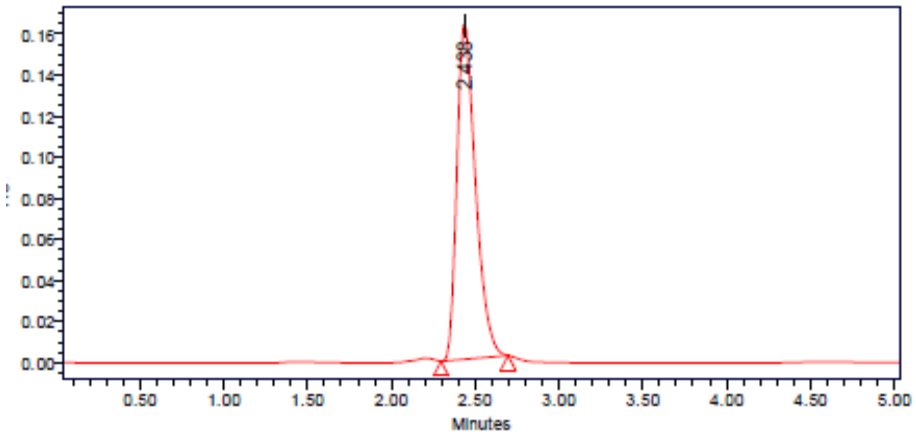


Table5: Results of repeatability for Capecitabine:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Capecitabine	2.436	1262901	164274	7295	138
2	Capecitabine	2.437	1265397	165899	5307	1.36
3	Capecitabine	2.438	1262726	166600	4347	1.40
4	Capecitabine	2.437	1261429	165280	7288	1.36
5	Capecitabine	2.436	1263264	166925	8324	1.35
Mean			1263143			
Std.dev			1437.481			
%RSD			0.113802			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

CONCLUSION:

The proposed RP-HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Capecitabine in bulk and in its Pharmaceutical dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and the % RSD values were with in 2 and the method was found to be precise. This method can be used for routine determination of Capecitabine in bulk and in Pharmaceutical dosage forms.

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