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RP-HPLC METHODE DEVELOPMENT AND VALIDATION FOR ESTIMATION OF "CAPECITABINE" AS API & DOSAGE FORM

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ABSTRACT

A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Capecitabine in tablet dosage forms. A C18column having 250 × 4.6 mm and mobile phase containing Methanol: Water (50:50v/v) was used. The flow rate was 1.0 ml/min and effluents are monitored at 241 nm. The retention time of Capecitabine is 3.15 min. The method was validated for specificity, linearity, accuracy, precision, limit of quantification, limit of detection, robustness in accordance with ICH guidelines. Limit of detection and limit of quantification for estimation of Capecitabine found to be 2.13 µg/ml and 6.46 µg/ml. Recovery of Capecitabine in tablet formulation was found to be 98.64%. Proposed method was successfully applied for the quantitative determination of Capecitabine in commercially available tablet dosage forms.

KEYWORDS: Capecitabine, RP-HPLC Method development, Validation.

INTRODUCTION

Analytical chemistry is defined as "The science and the art of determining the composition of materials, which deals with both theoretical, practical science. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species. Pharmaceutical analysis deals medicaments and their precursors. Quality is important in every product. Quality control is a concept, which strives to produce a perfect product. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Physico-chemical methods are

optical, photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Modern pharmaceutical analysis must need the following requirements. 1. The analysis should take a minimal time. 2. The accuracy of the analysis should meet the demands of Pharmacopoeia. 3. The analysis should be economical. 4. The selected method should be precise and selective. Chromatography: The term chromatography was first used by the Russian chemist and botanist Michael Tswett in 1906. The term chromatography is derived from the Greek words: Chroma for colour and Graphein to write. "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

Drug profile

Name: Capecitabine

Structure:

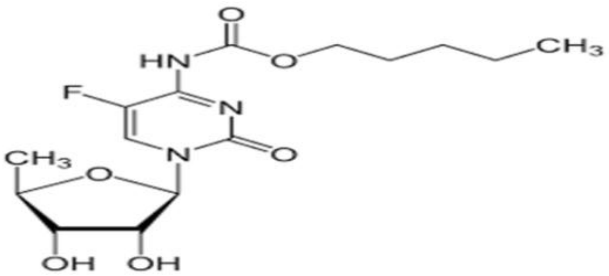

Figure 1: Structure of capacitabine.

Description

Capecitabine is prodrug which is enzymatically changed to 5- flurouracil in tumor and stop DNA synthesis there by reduce growth of the tumor tissue. It is 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.

IUPAC name: Pentyl N-{1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl}carbamate.

Chemical formula: $C_{15}H_{22}F_3N_3O_6$

Molecular mass: 359.35g/mol

Physical state: White to off white crystalline powder

Melting point: 110-121℃

Solubility: Water solubility is 26 mg/ml. It is soluble in ethanol, methanol and DMF.

pka: 5.41

$t_{1/2}$: 45-60 min

MATERIAL AND METHOD

Materials

Capecitabine obtained from swapnroop drug agency, Aurangabad.

Instrument

The analysis of the drug was carried out on thermofisher gradient system UV detector. Equipedv with C18 column (250 × 4.6 mm) and running chromoquest 4.1 software.

Selection of detection of wavelength

The UV spectrum of diluted solution of various concentration of Capecitabine in mobile phase was recorded using a UV spectrophotometer. The wavelength of maximum absorbance was observed at 241nm. This wavelength was used for detection of Capecitabine.

Preparation of Mobile phase

The aim is to find the correct concentration of the mobile phase. The mobile phase and its strength is a measure ability to pull analytes from the column. In reverse phase HPLC with aqueous mobile phases such as Methanol and water (50:50v/v). The retention time is also important criteria for selection of mobile phase.

Preparation of standard stock solution

An accurately weighed quantity pure powder of Capecitabine (25 mg) was transferred to 50ml volumetric flask dissolved and diluted to the mark with mixture of methanol and water in the ratio of 50:50. The volume was made up to the mark using same mixture of mobile phase to get final concentration 500µg/ml.

Preparation of sample solution

10 tablet label claim 500mg Capecitabine IP (CAPECAD, Ciplapharma ltd.) were weighed and crushed into fine powder. The amount of powder equivalent to 25mg of Capecitabine was weighed and transferred into the 50ml of mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for 20 min in two cycles each of 10 min. from the sample stock solution.

Optimized method for Capecitabine

Chromatographic condition

Parameter	Optimized Condition
Column	:C18 column(4.6m ×250mm)
Mobile phase	:Methanol: water (50:50)
Detection wavelength	:241nm
Flow rate	:1.0 ml/min
Column temperature	:Ambient
Sample size	:10µl
Run time	:6.0 min

System suitability testing

Preparation of working solution

From freshly prepared standard stock solution (500µg/ml), 1.0ml stock solution was pipetted out and diluted upto 10ml to obtain consequential solution of 50µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10min.Three replicatesof this solution were injected and result were recorded for RT, area, tailing factor, theoretical plates, SD, %RSD were calculated for the results and other parameters are shown in Table 1**.**

Method Validation

Linearity

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 Six concentrations is recommended by ICH guideline. The value of correlation co-efficient should fall around 0.99. The regression equation and correlation coefficient was calculated and found to be within the required limits as shown in Tables 2 and 3 respectively.

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 intra-day and inter-day precision results were shown in Table 4.

Accuracy/Recovery

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 recovery results for accuracy study of capecitabine were represented in Table 5.

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 of robustness study were shown in Tables 6 & 7 respectively.

Limit of detection and limit of quantification

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantification was calculated using following formula LOD= 3.3SD/S and LOQ= 10SD/S, where the SD= standard deviation of response (peak area) and S= slope of the calibration curve.

RESULT AND DISCUSSION

System suitability test

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, flow rate and the column were studied. The resulting chromatograms were recorded and the chromatographic parameters such as peak area, resolution and theoretical plates were integrated. The conditions obtained most excellent resolution; symmetry factor and theoretical plate were selected for further estimation.

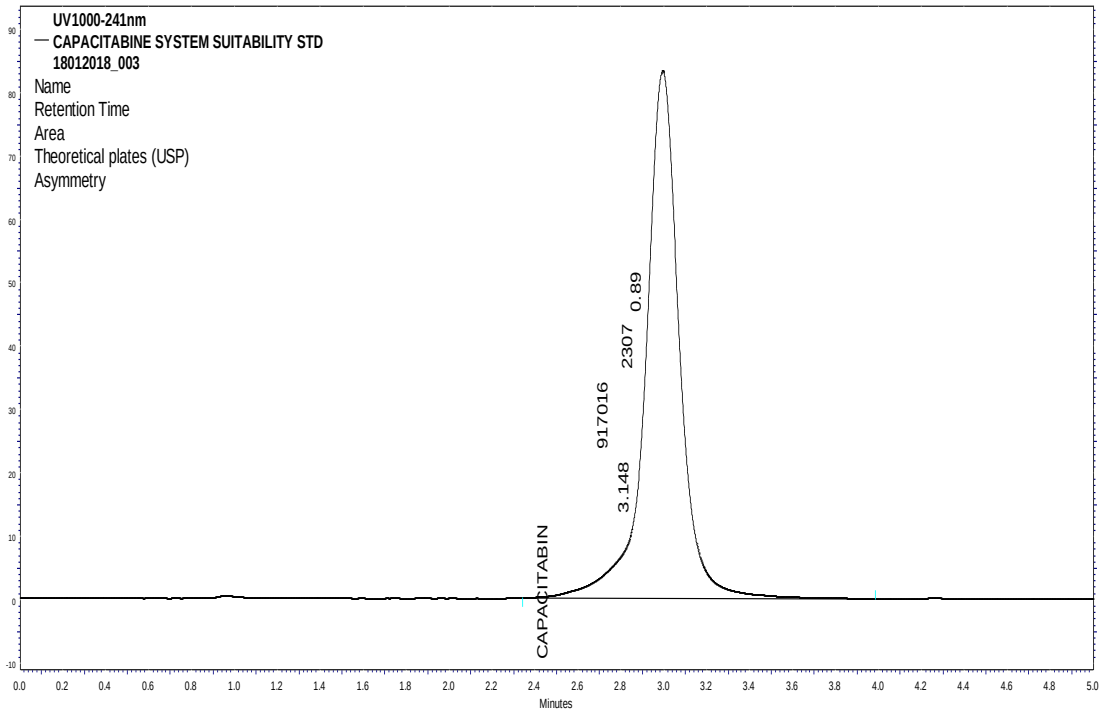

Fig. 1: Chromatogram of system suitability.

Table 1: System suitability parameter.

Sr.no	System suitability parameter	Mean observation	Standard limits	Inference
1	Retention time	3.15	NLT 2.0min	Passed
2	Area	916877	NLT 2000	Passed
3	Theoretical plate	23265	NLT 2000	Passed
4	Tailing factor	0.88	NMT 2.0	Passed
5	% RSD	0.59	NMT2.0%	Passed

Linearity

The standard calibration curve was constructed between concentration Vs peak area and linearity was found in the range from 12.5µg/ml to 75µg/ml. The regression equation and correlation coefficient was calculated and found to be within the required limit.

Table 2: Linearity data of Capecitabine.

Sr. no.	Conc. µg/ml	Mean Area*
1	12.5	229123
2	25	447707
3	37.5	668099
4	50	911802
5	62.5	1157138
6	75	1363193

Table 3: Results for Linearity.

Parameters	Values
Concentration range	12.5µg/ml to 75µg/ml
Regression equation (Y)	y = 18383x - 8057.
Slope (m)	18383
Intercept (c)	-8057.7
Correlation coefficient	0.9994

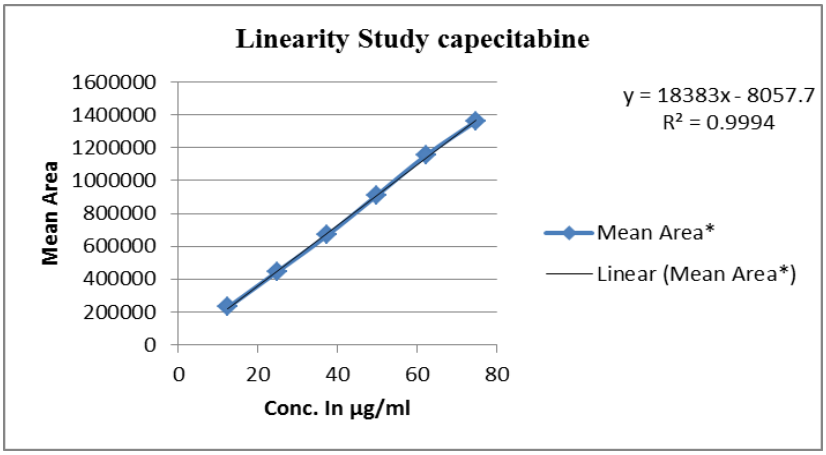

Fig. 2: Calibration curve of Capecitabine.

Precision

Precision Intra-day precision was investigated by replicate applications and measurements of peak area for Capecitabine for three times on the same day under similar conditions. Inter-day precision was obtained from %RSD values obtained by repeating three times on two different days. The %RSD was calculated which was within the acceptable limits of not more than 2.0.

Table 4: Results of Precision.

Conc.(µg/ml)	Intra-day precision		Inter-day Precision	
	Mean± SD	% RSD	Mean± SD	% RSD
20	378830.7±859	0.23	379653±1778.1	0.47
35	645733±5353.45	0.83	345609±1897.8	0.29
70	1320003±2502.4	0.13	1323303±1858.4	0.22

Accuracy/ Recovery

The accuracy of the method was tested by triplicate sample at 3 different concentrations equivalent to 75%, 100% and 125% of the active ingredient, by adding a known amount of Capecitabine standard to a sample with predetermined amount of Capecitabine. The recovered amount of Capecitabine, % recovery of each concentration was calculated to determine accuracy.

Table 5: Results of Accuracy/ Recovery.

Sr No	% Recovery Level	Amount of Standard Taken (µg/ml)	Amount of Sample Spiked (µg/ml)	Mean Area*	Amount Recovered (µg/ml)	% recovery
1	80	50	40	1652664	90	100.37
2	100	50	50	1805337	100	98.64
3	120	50	60	2012869	110	99.94

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were the composition of flow rate, and mobile phase composition. The results of robustness study indicated that the small change in the conditions did not significantly affect the determination of Capecitabine.

Table 6: Results for Robustness Mobile phase composition variation.

Sr.no	Concentration (µg/ml)	Area	
		45:55 v/v Methanol: water	**55:45 v/v Methanol: water**
1		905162	901659
2	50 µg/ml)	905007	901721
3		910107	903821
%RSD		**0.32%**	**0.14%**

Table 7: Results for Robustness flow rate variation.

Sr. no	Concentration (µg/ml)	Area	
		0.95 (ml/minute)	**1.05 ml/minute)**
1		909370	888066
2	50 µg/ml	906987	903477
3		913673	901130
%RSD		**0.37%**	**0.93%**

Limit of detection and Limit of quantification

The Limit of detection was found to be 0.42µg/ml

The Limit of quantification was found to be 1.27µg/m

CONCLUSION

The proposed method for the assay of Capecitabine was simple, rapid, accurate, precise, sensitive and economic for the quantification of Capecitabine from its pharmaceutical dosage forms. The method was validated for linearity, accuracy, precision, LOD, LOQ, robustness and system suitability. The method was free from interference of other active ingredient and excipients. Hence it can be concluded that this method may be employed for routine quality control analysis of capecitabine in Active pharmaceutical ingredient and Formulation product.

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