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RP-HPLC method development and validation of capecitabine in bulk drug and formulation

G.Chaitanya, G.Venkata Ramana, A. K. M. Pawar*

Department of Pharmaceutical Analysis, A. U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

***Corresponding Author: A.K.M.Pawar**

Email ID: akpawar.pharm@auvsp.edu.in

ABSTRACT

Objective

To Develop and validate a simple, selective, rapid, precise and accurate High Performance Liquid Chromatographic Method for determination of capecitabine in bulk and its pharmaceutical formulation product.

Method

RP-HPLC method was performed by using a mobile phase consisting mixture of Methanol and Ammonium acetate buffer (p^H 4.5) in the proportion 60:40v/v. A ZORBAX Eclipse plus C_{18} (4.6 ×100mm, 3.5µ) column was used as a stationary phase. . HPLC analysis of Capecitabine was carried out at a wavelength of 241nm with a flow rate of 1ml/min.

Results

The linear regression analysis data for the calibration curve showed a good linear relationship with a correlation coefficient 0.9984. The linear regression equation was

y=3726540. 2 x+27390388. 1. This was found to give a sharp peak of Capecitabine at a retention time of 2.77min.Validation parameters were evaluated for the method according to the ICH (Q2R1) guidelines. The limit of detection and limit of quantification for the method were 0.6721µg/mL and 1.9989µg/mL, respectively. The %RSD values for Intra-day precision and Inter-day precision were found to be 0.31 % and 0.30% respectively. Accuracy of the method was determined through recovery studies which were found to be within 97.57-102.22%.

Conclusion

The method was validated for system suitability, accuracy, precision, robustness and ruggedness. The precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better than the earlier methods developed for the quantification of Capecitabine.

Keywords: Capecitabine, RP-HPLC Method Development, Validation.

INTRODUCTION [1,2]

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 tumor-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 tumor 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. The literature survey revealed that several HPLC methods for determination of Capecitabine were reported, but with longer retention time. Since the retention time is one of the important factors for the separation of drugs using HPLC, the present work reports the development and validation of an HPLC method for the estimation of Capecitabine in bulk and formulation with shorter retention time and the method has been highly used for its sensitivity and reproducibility.

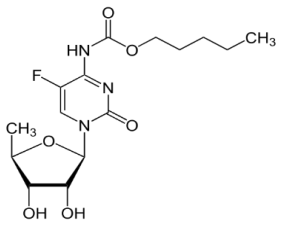

Fig 1: Structure of Capecitabine [3, 4]

MATERIALS AND METHODS

Instruments

HPLC Agilent 1260 with PDA detector & Auto sampler, Zorbax eclipse Plus C_{18} (4.6 ×100mm, 3.5µ) equipped with Open lab EZ Chrome software, Lab, India – T60 UV/Vis double beam spectrophotometer, Mettler Toledo ME 204 balance, Eutech p^H 700 p^H meter.

Chemicals and Reagents

The sample of capecitabine standard was a gift sample from MSN Laboratories Ltd, Hyderabad, India. HPLC grade Methanol, water and Ammonium acetate were procured from Merck Pharmaceuticals Private Ltd., Mumbai, India. Glacial acetic acid was also used.

Selection of detection wavelength

The UV spectrum of diluted solutions of various concentrations 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 content of the Mobile Phase was prepared from filtered and degassed mixture in the ratio 60:40v/v of methanol and ammonium acetate buffer (1.54gm of Ammonium acetate in 1.0 litre distilled Water) and p^H was adjusted to 4.5 with 0.2M glacial acetic acid.

Preparation of Capecitabine Standard Stock solution

100mg of Capecitabine powder was weighed and transferred into 100ml volumetric flask and 30ml of diluent was added to it, sonicated and was further filtered through 0.45µ filter paper and the volume was made up with diluent. Then 100µg/mL working standard solution was prepared by pipetting out 10 ml of 1000µg/mL solution into the 100ml volumetric flask and the remaining volume was made up with diluent.

Preparation of Sample solution

10 tablets, each containing 500mg of Capecitabine were weighed and crushed into fine powder and the amount of powder equivalent to 100mg of Capecitabine was weighed and transferred into 100ml dried volumetric flask. The content was dissolved by adding 30ml of diluent and rapidly shaking for a few minutes. The volume was made up with diluent, mixed well and injected immediately.

Optimised method for Capecitabine

Chromatographic Conditions

Column : Zorbax Eclipse Plus C_{18} (4.6×100mm, 3.5µ)
Mobile phase : Methanol: Ammonium acetate buffer- p^H 4.5 (60:40)
Flow rate : 1 ml /min
Wavelength : 241nm
Column temperature : 25°C
Injection volume : 15µL
Run time : 5 minutes
Diluent : Methanol: Water (60:40)
Elution : Isocratic
Needle wash : Methanol

METHOD VALIDATION [5-11]

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 five 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 1 and 2 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 Tables 3 and 4 respectively.

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.

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.

Robustness

The robustness of an analytical procedure are 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 and 8 respectively

RESULTS AND DISCUSSION

Linearity

The standard calibration curve was constructed between concentration V_s peak area and linearity was found in the range from 20µg/mL to 100µg/mL. The regression equation and correlation coefficient was calculated and found to be within the required limit.

Table 1: Linearity parameters and their values

S. No	Concentration	Area
1	20	979004765
2	40	179504390
3	60	257038920
4	80	320327053
5	100	400143166

Table 2: Results for Linearity

S. No	Parameters	Values
1	Concentration range	20-100µg/mL
2	Regression equation (Y)	Y=3726540.2X+27390388.1
3	Correlation coefficient(r^2)	0.998
4	Slope(m)	3726540.2
5	y-intercept(c)	27390388.1

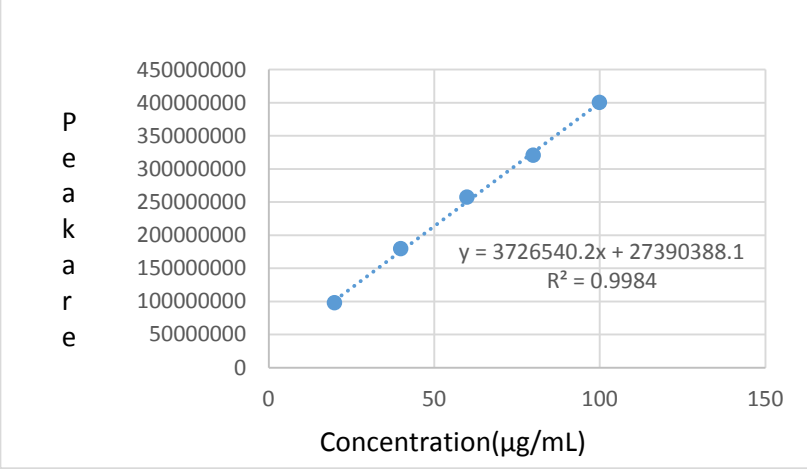

Fig.1: Calibration curve of Capecitabine

Precision

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

Table 3: Intra-day Results for Precision

Concentration(µg/mL)	Injection no.	Area	%RSD
60(µg/ml)	1	257038920	0.31%
	2	258212471	
	3	256126510	
	4	257201546	
	5	257003481	
	6	256021567	

Table 4: Inter-day Results for Precision

Concentration(µg/ml)	Injection no.	Area	%RSD
60(µg/ml)	1	257124852	0.30%
	2	258569842	
	3	256521436	
	4	257254216	
	5	257042157	
	6	256367421	

Accuracy

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 pre-determined amount of Capecitabine. The recovered amount of Capecitabine, %RSD of recovery, % recovery of each concentration was calculated to determine the accuracy.

Table 5: Results for Accuracy

Sample. No	% Level	Standard amount	Spiked amount	Amount found	% Recovery	Mean Recovery
1.	75 %	80	60	61.32	102.20	Mean=102.221
2		80	60	61.34	102.22	SD = 0.0180
3		80	60	61.34	102.24	%RSD=0.02%
1	100 %	80	80	79.99	99.98	Mean = 99.99
2		80	80	80	100	SD = 0.0115
3		80	80	80	100	%RSD=0.01%
1	125 %	80	100	99.57	97.57	Mean = 97.57
2		80	100	99.58	97.58	SD = 0.0057
3		80	100	99.57	97.57	%RSD=0.01%

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, p^H and mobile phase. 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

S. No	Concentration (µg/mL)	Area

		0.8(ml/minute)	1.2(ml/minute)
1		257038916	257135915
2		254217540	257217540
3		257982451	256824524
4	(60µg/mL)	25824503	256214500
5		255124901	255524907
6		257412031	257612034
%RSD		0.63%	0.30%

Table 7: Results for Robustness p^H variation

S. No	Concentration (µg/mL)	Area	
		p^H 4.3	p^H 4.7
1		257412820	254871230
2		254871245	258746950
3		256485124	257481245
4	(60µg/mL)	251538546	254132549
5		252487120	253157245
6		259124850	259854721
%RSD		1.15%	1.06%

Table 8: Results for Robustness Mobile phase composition variation

S.no	Concentration (µg/mL)	Area	
		65:35 v/v Methanol: Buffer	75:25 v/v Methanol: Buffer
1		254745147	257481247
2		256487120	257421510
3	(60µg/mL)	258694721	256854214
4		257124573	253142658
5		257654801	258451270
6		256124523	256140124
%RSD		0.53%	0.72%

Limit of detection and Limit of quantification

The Limit of detection was found to be 0.6721µg/mL

The Limit of quantification was found to be 1.9989µg/mL

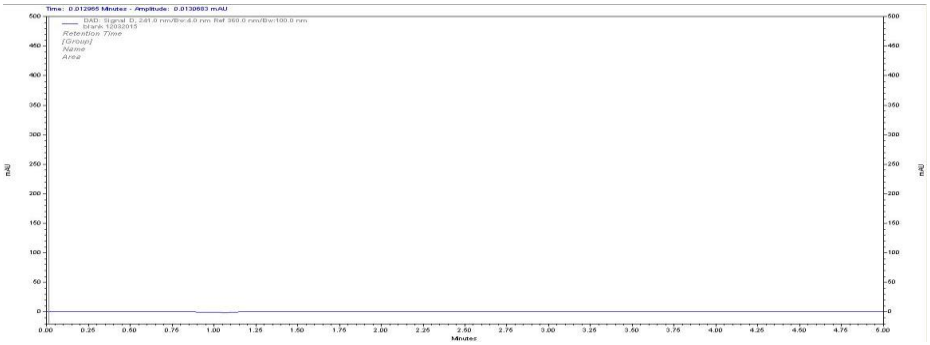

Fig: 2 Blank chromatogram of capecitabine.

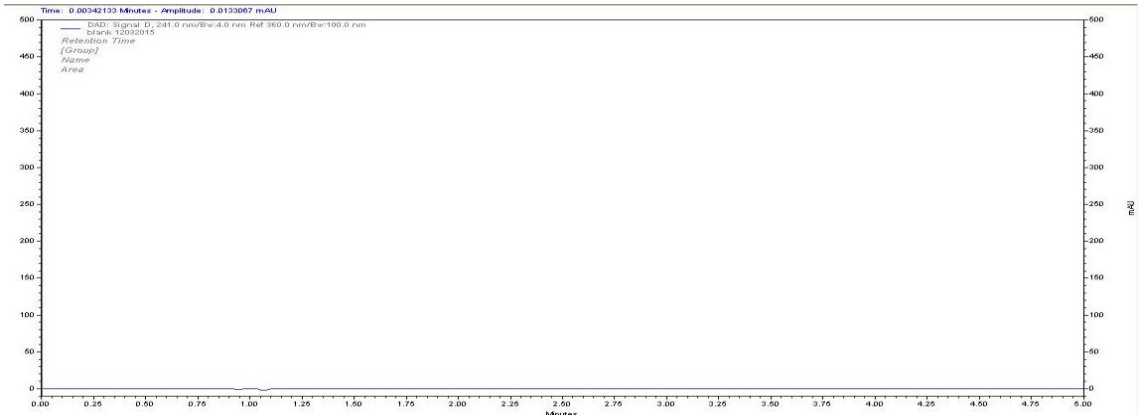

Fig: 3 chromatogram of placebo

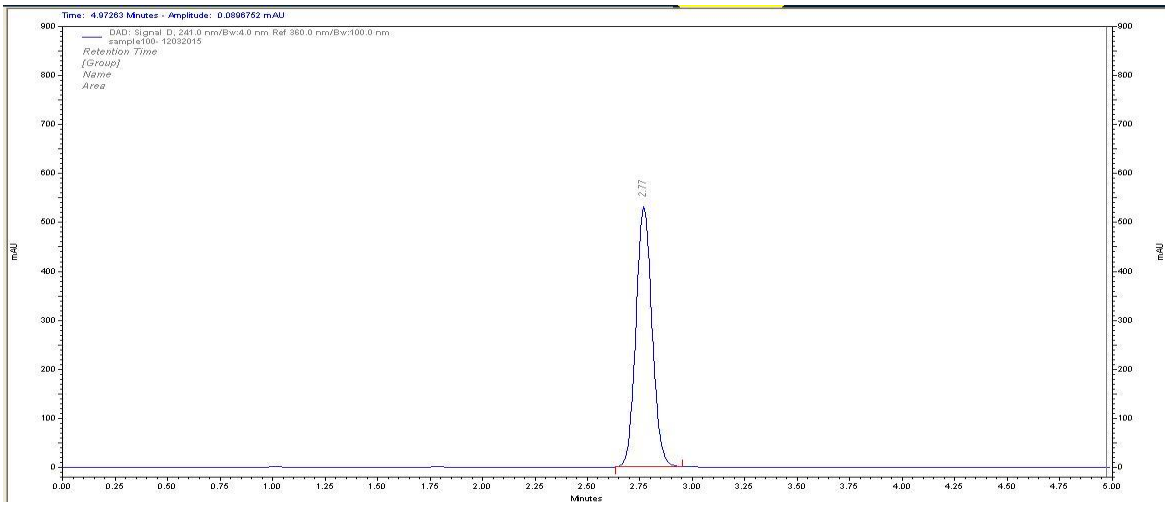

Fig: 4 Standard chromatogram of capecitabine

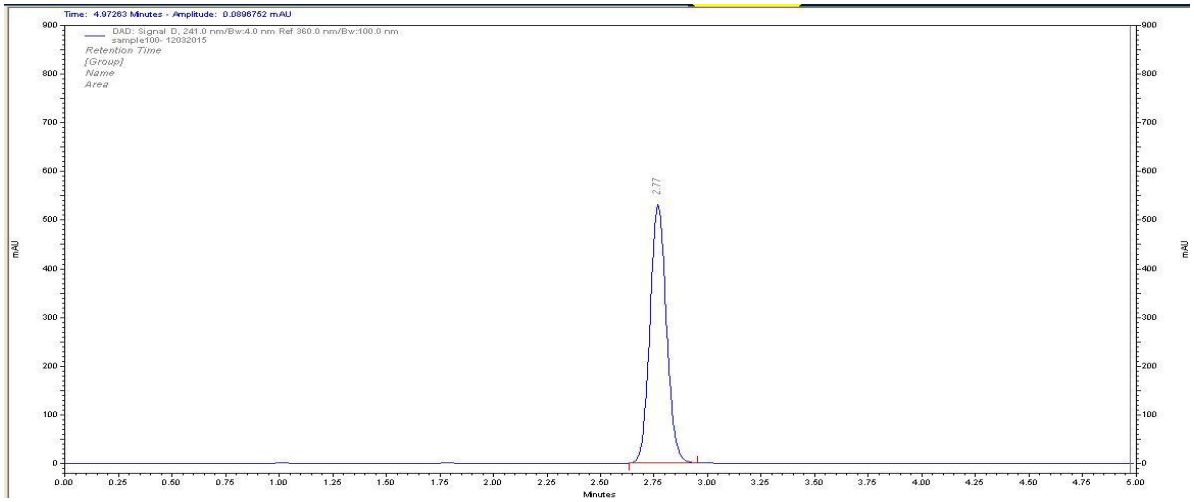

Fig: 5 Formulation chromatogram of capecitabine

CONCLUSION

The proposed method for the assay of Capecitabine was rapid, accurate, precise and sensitive 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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REFERENCES

[1]. P. Ravisankar, G.Devala Rao, M. Naveen Kumar; An Improved RP-HPLC method for the quantitative determination of capecitabine in bulk and pharmaceutical tablet dosage form; Scholars research Library; 2013
[2]. Fathima Anjum, Tangadpally Radhika, Kondepudi R.Kumari, Makula Ajitha , International research journal of pharmacy;2012;Issue No.2230-8407;177-180 http: // www.drugbank.com
[3]. http: // www.usfda.com
[4]. Nash R. A.: Pharmaceutical process validation, An International 3rd Edition Revised and expanded; Vol.129, 507- 522.
[5]. Gurudeep R. Chatwal : Instrumental Methods of Chemical Analysis; 2.566-2.699
[6]. ICH, Q2B, Harmonised Tripartite Guideline, Validation of Analytical procedure: Methodology, IFPMA, in: proceedings of the International Conferencce on Harmonisation, Geneva, March 1996.
[7]. Liod R. Snyder: Practical HPLC Method Development; 2nd edition .
[8]. Narendra Devanaboyina, Y.Sai Kishore, P.Pushpalatha; Development and Validation of New RP-HPLC method for Analysis of capecitabine in Pharmaceutical dosage form; International journal of science inventions Today,2013.
[9]. Sangitha N.Waghule, Nitin P.jain; method development and validation of HPLC method for Determination of Azithromycin; Scholars research Library,2013,5(4), 166-172.
[10]. P.Ravisankar, G.Devala Rao; Development and Validation of RP-HPLC method for determination of Levamisole in bulk and dosage form; Asian journal of Pharmaceutical and Clinical research; Vol 6, suppl 3, 2013.