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Method Development and Validation of Capecitabine in Tablets by RP-HPLC

Shaik Yasmeen*, Karavadi Thejomoorthy and Ch. Saibabu

Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Kanumalla, Singarayakonda-523101

ABSTRACT

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of capecitabine in Bulk and its pharmaceutical formulation. Separation was achieved with a Develosil ODS-MG-5 ; 100 x 4.6mm I.D; particle size 5 µm)) Column and Acetate buffer, using buffer and Methanol (450:550) v/v as eluent and purified water, methanol and acetonitrile(600:350:50)v/v as diluent at flow rate 1.0 mL/min and the Column temperature was 40°C. UV detection was performed at 250 nm and sample temperature was maintained at 5°C. The method is simple, rapid, and selective. The described method of Capecitabine is linear over a range of 6 µg/mL to 30 µg/mL. The method precision for the determination of assay was below 2.0% RSD. The method enables accurate, precise, and rapid analysis of capecitabine. It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

Keywords: Capecitabin, RP-HPLC, Method development, Method validation.

Introduction:

Capecitabine, is chemically known as 5'-deoxy-5-fluor[(pentyloxy)carbonyl]-cytidine.. 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 & Methods:

Instrument:

The equipments used are double beam Uv-Visible spectrophotometer (Make: Schimadzu) equipped with Uv probe software, Micro balance (Make: Mettler Toledo), pH meter (Make: Lab India), Sonicator: Ultrasonic bath sonicator (Make: SV Scientific), centrifuge (Make: SV Scientific). The chromatographic separation was carried out using HPLC Alliance Waters (2487) equipped with gradient system, connected to dual absorbance detector. The data was acquired by Empower Pro.

Chemicals & Reagents:

Capecitabin working standard was used for analysis. Methanol, Acetonitrile used are of HPLC grade solvents. Chemicals Glacial acetic acid are of AR grade.

METHOD DEVELOPMENT

I. Solubility studies:

Solubility studies for Capecitabine revealed the solubility of drug in methanol. capecitabine was slightly soluble in water.

II. Selection of detector wave length:

An UV spectrum of 30 µg / ml Capecitabine in diluents (purified water: Methanol: ACN) in (600:350:50) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength of 250 nm was selected. At this wavelength Capecitabine standard showed good absorbance. For spectra refer fig.1. For optimized chromatogram refer fig.2.

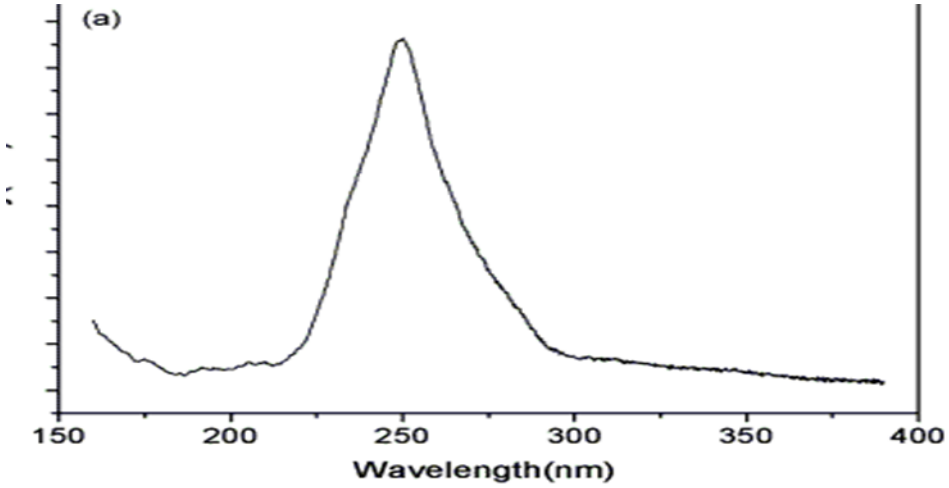


Fig.no.1 Spectra of capecitabine showing λ max of 250nm

Optimized Chromatographic conditions:

Mobile phase	**:** Buffer (glacial acetic acid) and Methanol the ratio of(450:550)
Column	**:** Develosil ODS- MG-5(100x4.6mm), 5µm
Flow rate	**:** 1.0 ml/min
Detector wavelength	**:** 250 nm
Column temperature	**:** 40^0C
Injection volume	**:** 10 µl
Run time	**:** 10 min
Retention time	**:** 5.334 min

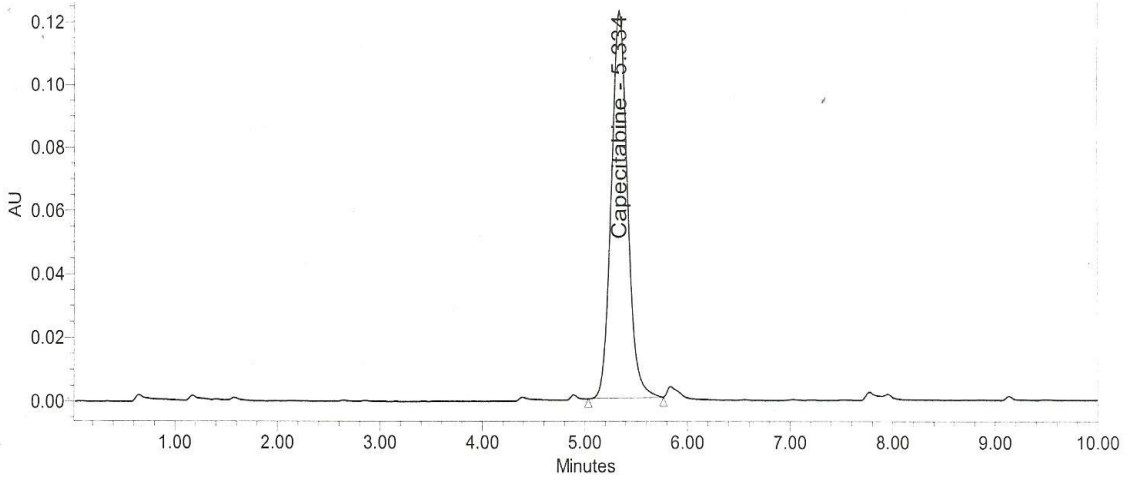


Fig. No. 2 Optimized Chromatogram

Observation: Good peak was obtained.

Preparation of solutions:

Buffer Preparation:

Accurately transfer 1 mL of Glacial Acetic acid in 1000 ml of purified water and mix. pH need not to be adjusted.

Mobile phase

Prepare a filtered (0.45µ*)* and degassed mixture of buffer , Methanol in the ratio of 450:550 v/v respectively

Diluent

Prepare a mixture of purified Water, Methanol and Acetonitrile in the ratio of 600:350:50 v/v respectively.

Preparative Steps for Assay method development:

Standard Preparation:

Accurately Weigh and transfer accurately 60mg of Capecitabine working Standard into a 1000 ml clean dry volumetric flask, and add about 600 ml of diluents, and sonicate to dissolve. cool the solution to room temperature and dilute to volume with diluents and mix.

Sample preparation:

Accurately weigh and transfer the sample equivalent to 15 mg of Capecitabine into a 250ml Amber colour volumetric flask. Add about 180 ml of diluents, shake for 10 minutes on orbital shaker and sonicate for 20 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with diluents . filter the solution through 0.45µm PVDF filter.

METHOD VALIDATION

This Validation describes the procedure for assay of Capecitabine tablets by HPLC as per ICH Guidelines (Q2B). The method validation parameters for assay of Capecitabine monohydrate include

- Specificity
- System Suitability
- Accuracy
- Linearity and Range
- Precision

1. Intermediate precision (ruggedness)
2. Method precision

- Detection Limit
- Quantitation Limit
- Robustness

VALIDATION

SYSTEM SUITABILITY:

The system suitability studies were done with the 60mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The chromatogram is recorded and are shown in fig. No.3 and Table. No.1&2.

Fig.No.3 Chromatogram showing system suitability

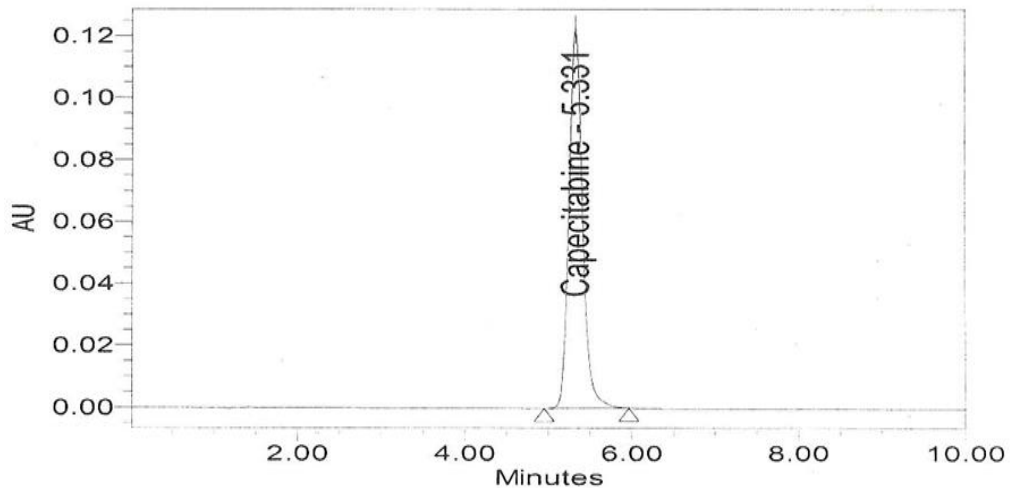


Table No.1. Showing results from system suitability study

S. No	Peak Name	Rt (min)	Area	USP Tailing	Plate count
1	Capecitabine	5.332	1383340	1.12	5413
2	Capecitabine	5.331	1387644	1.12	5377
3	Capecitabine	5.330	1387750	1.11	5396
4	Capecitabine	5.330	1388970	1.11	5385
5	Capecitabine	5.330	1389243	1.11	5369
6	Capecitabine	5.328	1385820	1.12	5364
Mean			1387128	1.12	5384
SD			2217.27		
%RSD			0.16		

Table No.2. Summary of system suitability study

System suitability parameters	Results (avg)
%RSD	0.16
Tailing factor	1.12
Plate count	5384
No. of theoretical plates	4890
Relative retention	…….
Resolution	…….
Capacity factor	…….

LINEARITY

The linearity study was performed for the concentration of 6µg/ml to 30µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table. No.3.

Table No.3 showing results from linearity study

S. No	Linearity Level	Concentration (µg/ml)	Peak area
1	I	6	143119
2	II	12	282164
3	III	18	432216
4	IV	24	572315
5	V	30	692418
Correlation Coefficient			**0.999**

The linearity study was performed the correlation coefficient of capecitabine was found to be 0.999 respectively (NMT 0.999).

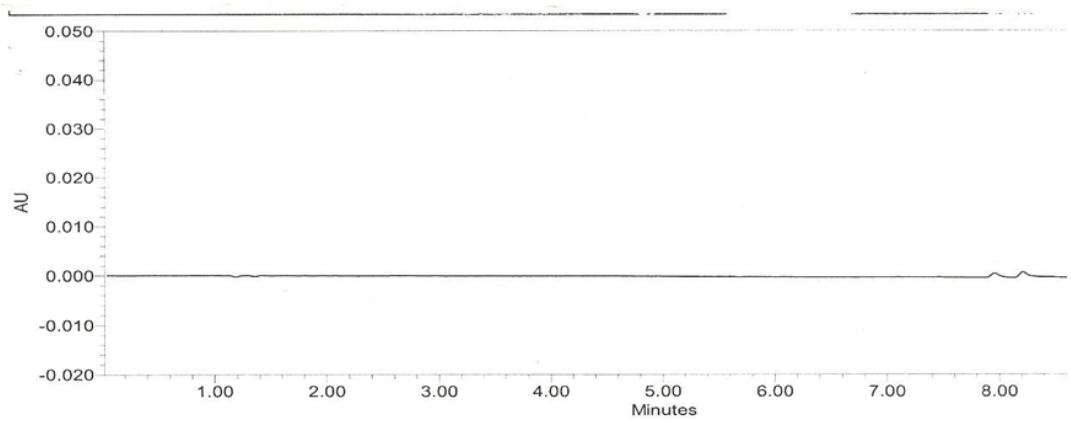
0.999 respectively (NMT 0.999).

SPECIFICITY

The system suitability for specificity was carried out to determine whether there is any interferences of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig. No.4&5

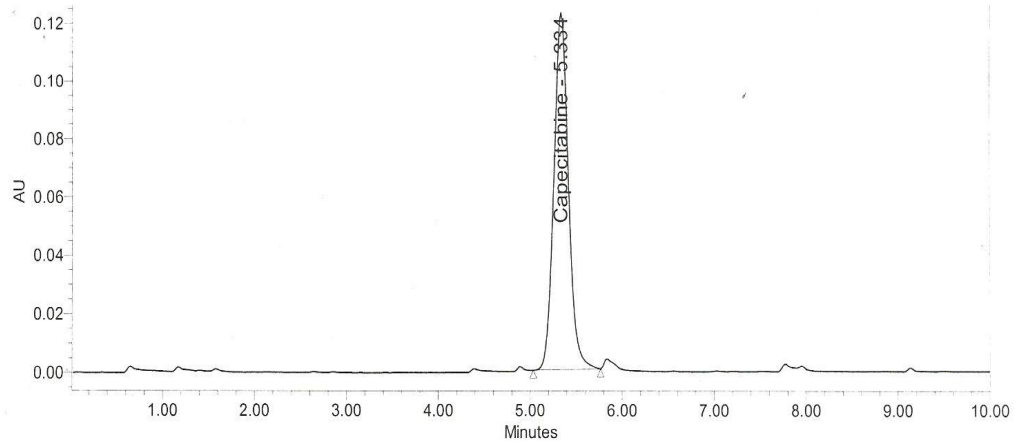
Blank:

Fig No.4 Chromatogram showing blank preparation



Capecitabine standard:

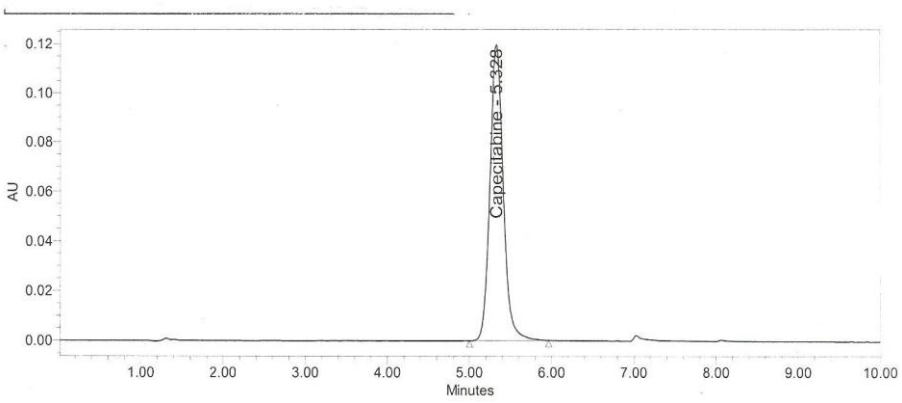
Fig No.5 Chromatogram showing standard preparation



S. No	Drug name	vail	RT	Peak area	USP plate count	USP tailing
1.	capecitabine	5	5.334	1364432	5431	1.10

Capecitabine sample:

Fig No.6 Chromatogram showing sample preparation



	Drug name	vail	RT	Peak area	USP plate count	USP tailing
1.	capecitabine	6	5.328	1356532	5431	1.10

The specificity test was performed for Capecitabine. It was found that there was no interference of impurities in retention time of analytical peak. The method shows excellent specificity with capecitabine eluting at retention of 5.328 minutes. No interference was observed with mobile phase.

ACCURACY

The accuracy study was performed for 50%, 100% and 150% for capecitabine. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. Results are tabulated in Table. No.4.

Table No.4. Showing result from accuracy study

Level of % recovery	**Amount of drug spiked(µg/ml)**	**Drug recovered**	**%Recovery**	**Mean**	**SD**	**%RSD**
80	9.6	9.62	100.2	100.4	0.346	0.34
		9.62	100.2			
		9.68	100.8			
100	12	12.23	101.9	101.6	0.974	0.95
		12.08	100.6			
		12.31	102.5			
120	14.4	14.26	99.02	99.70	0.6451	0.64
		14.21	99.8			
		14.45	100.3			

The accuracy study was performed for % recovery. The % recovery was found to be 100.4 to 99.70% respectively. (NLT 98% and NMT 102%).

PRECISION

- Repeatability
- Intermediate Precision

Repeatability

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

Intermediate Precision/Ruggedness

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

Repeatability

The precision study was performed for six injections of capecitabine. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD. Results are tabulated in Table.Nos.5&6.

Table No.5. Showing from precision study-repeatability (60 µg/ml)

Method Precision

S. No	**Peak Name**	**Peak area**
1	Capecitabine	1381620
2	Capecitabine	1384273
3	Capecitabine	1382656
4	capecitabine	1383288
5	Capecitabine	1388610
6	Capecitabine	1382144
Mean		1383765
SD		1502.76
%RSD		0.10

Table No.6. Showing from precision study-repeatability(60µg/ml)

System Precision

S.No	**Peak Name**	**Peak area**
1	Capecitabine	1382136
2	Capecitabine	1385243
3	Capecitabine	1386230
4	Capecitabine	1386790
5	Capecitabine	1384273
6	Capecitabine	1385280
Mean		1384992
SD		1648.33
%RSD		0.12

Ruggedness

Intra-day precision

Intra-day precision was carried out on same day, same HPLC system, using same column at different times. Calculated average area and %R.S.D for 12 tests, (Condition I and Condition II).

Inter-day precision

Inter-day precision was carried out on same HPLC system, using same column on another day.

The average area was calculated and %R.S.D. for 6 replicate injections of standard drug solutions.

Method precision: Six Sample solutions are prepared as per test method and injected as per test procedure.

Table No.7 . Showing from precision study- Intraday

Conc µg/ml	**Peak area**	**Statistical parameters**
40	912546	Mean:915887
	916382	S.D:3123.5
	918734	%R.S.D:0.34
60	1364876	Mean:1366257
	1366208	S.D:1407.15
	137689	%R.S.D:0.10
80	1814786	Mean:1816049
	1816124	S.D:1227.72
	1817238	%R.S.D:0.06

Table No.8. Showing from precision study- Interday

Conc µg/ml	**Peak area**			**Statistical parameters**
	Day-1	**Day-2**	**Day-3**	Mean:915780
40	912436	916257	918648	S.D:3133.3
				%R.S.D:0.34
60	1364926	1365182	1367394	Mean:1365834
				S.D:1357.0
				%R.S.D:0.09
80	1814954	1816242	1817438	Mean:1816211
				S.D:1242.28
				%R.S.D:0.07

The precision of method was determined by replicate injection of sample solution. The %RSD of area of intraday precision are 0.3%, 0.10% and 0.06%. %RSD of interday precision was found to be 0.3%,0.09% and 0.07%. Precision results are within the limits. (NMT 2)

LIMIT OF DETECTION AND QUANTIFICATION

Detection Limit

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualtitated as an exact value

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank = 42.43 µV

Signal Obtained from LOD solution = 0.00948 µV

LOD=3.3×σ/s= 3.3×0.00948/42.43=0.000737

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank = 42.43 µV

Signal Obtained from LOQ solution = 0.00948 µV

LOD=10×σ/s= 10×0.00948/42.43=0.02342

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

ROBUSTNESS

The robustness of an analytical method 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. Robustness was done by changing the flow rate (± 1), column temperature (± 5°C), Changing the wavelength (± 5 nm). The %RSD of peak area, tailing factor and theoretical plates of Capecitabine standard was found within the limits.

Table No.9. Showing results from robustness study

Replicate standard injections at 0.9ml\min			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364216	Average :1364003 % RSD = 0.11	% RSD : not more than 2%
2	1362325		
3	1365470		

Table No.10. Showing results from robustness study

Replicate standard injections at 1.1ml\min			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1384273	Average :1385179 % RSD = 0.14	% RSD : not more than 2%
2	1388610		
3	1382656		

The analytical method was found to be robust with respect to change in flow rate.

Influence on variation of Column Temperature:

Table No.11. Showing results from robustness study

Replicate standard injections at 35^0c			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364354	Average :1366608 % RSD = 0.10%	% RSD : not more than 1%
2	1367124		
3	1368346		

Table No.12. Showing results from robustness study

Replicate standard injections at 45^0c			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364592	Average :1366723 % RSD = 0.15%	% RSD : not more than 1%
2	1366831		
3	1368746		

Influence on variation of wave length :

Table No.13. Showing results from robustness study

Replicate standard injections at wave length 245 nm			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1364234	Average :1364798 % RSD = 0.04	% RSD : not more than 1%
2	1365173		
3	1364986		

Table No. 14. Showing results from robustness study

Replicate standard injections at wave length 255nm			
Injection No	**Peak area**	**Observation**	**Acceptance criteria**
1	1365216	Average :1366677 % RSD = 0.09	% RSD : not more than 1%
2	1366528		
3	1368287		

Assay calculation-:

$$\%\text{Assay} = \frac{TA}{SA} \times \frac{SW}{100} \times \frac{250}{\text{TW}} \times \frac{P}{100} \times \frac{Avg.wt}{LA} \times 100$$

%Assay = 99.32%

CONCLUSION

A new method has been established for estimation of Capecitabine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Capecitabine by using Develosil ODS-MG-5 column, flow rate was 1.0ml/min, mobile Phase: Buffer and Methanol (450:550v/v) and Diluent mixture of purified water, Methanol and Acetonitrile (600:350:50). Detection wave length was 250nm.The instrument used was WATERS HPLC auto sampler. The retention times were found to be 5.334 mins. The analytical method was validated according to ICH guidelines (ICH Q2b). The correlation coefficient (r^2) was found to be 0.999, % recovery was 100.4-99.70% and %RSD for precision on replicate injection was 0.10 and intermediate precision for intraday precision at condition-I,II and III was 0.3, 0.10 and 0.06 % interday precision at condition-I,II and III was 0.3,0.09 and 0.07% respectively. The precision study was precise, robust, and repeatable. LOD value was 0.000737 and LOQ value was 0.02342. Hence the method can be used for routine analysis of capecitabine in API and tablet dosage form.

References:

1) Willard HH, Merritt LL, Dean JA and Settle FA, (2001), **Instrumental Methods of Analysis,** 7th ed., CBS Publishers and Distributors, Delhi, p.3.

2) Skoog DA, West DM and Holler FJ, (1996), **Fundamentals of Analytical Chemistry,** 7th ed., Saunders College Publishing, Philadephia, p. 1-3.

3) Sharma BK, (2002), **Instrumental Methods of Chemical Analysis**, 21st ed., Goel Publishing House, Meerut, p. 3-5.

4) Skoog DA, Holler FJ, Timothy A and Niemann NW, (2004), **Principle of Instrumental Analysis**, 5th ed., Eastern Press, Bangalore, p. 1-2, 678-688, 695-696.

5) Scott RPW, (2003), **Technique and Practice of chromatography,** Marcel Dekker, New York, Vol. 70, p. 1-12.

6) Jeffery GH, Basset J, Mendham J and Denney RC, (1996), **Vogel's textbook of Quantitative Chemical analysis,** 5th ed., Longman Publication, England, p. 647-649.

7) Connors KA, (1999), **a textbook of Pharmaceutical Analysis,** 8th ed., Wiley-Interscience, New York, p. 408-421.

8) Hamilton RJ and Sewell PA (1982) **Introduction to HPLC,** 2nd ed., Chapman and Hall, London, p. 189.

9) Snyder LR, Kirkland JJ and Glajch JL, (1997), **Practical HPLC Method Development**, 2nd ed., New York, Wiley, p. 1-20.

10) Sethi PD, (2001), HPLC **'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulations,** 1st ed., CBS Publishers and Distributors, New Delhi, p. 3-11, 116-120.

11) Munson JW, (2001), **Pharmaceutical Analysis: Modern Methods (Part B)**, Marcel Dekker, New York, p. 51-54,120,175.

12) Scott RPW, **Liquid Chromatography for the Analyst**, Marcel Dekker, New York, Vol. 67, p. 15-23, 265-272.

13) Chatwal GR and Anand (2001) SK, (2004), **Instrumental Methods of Chemical Analysis,** 5th ed., Himalaya Publishing House, Delhi, p. 2.599-2.605.

14) Ewing's, Analytical Instrumentation Handbook,(2005) 3rd ed., edited by Cazes J, Marcel Dekker, New York, p. 995-998.

15) Sharma BK, (2003), **Instrumental Methods of Chemical Analysis**, 25th ed., Goel Publishing House, Meerut, p. 39-42, 96-104.

16) Parimoo P, (1998), **Pharmaceutical Analysis,** 1st ed., CBS Publication and Distributors, New Delhi, p. 151-152.

17) Schrimer RE, (1991), **Modern Method Pharmaceutical Analysis,** 2nd ed., CRC Press, Vol.-1, p. 75-76.

18) Beckett AH and Stenlake JB, (2004), **Practical Pharmaceutical Chemistry,** Part 2, CBS Publishers and Distributors, New Delhi, p. 282-283

19) ICH Harmonised Tripartite Guideline (Nov 2005) **Validation of Analytical Procedures:** Text and Methodology Q2 (R1).

20) ICH, (October 1993), Q1A **Stability testing of New Drug Substances and Products,** In: Proceedings of the International Conference on Harmonization, Geneva.

21) Liia D. Vainchtein, Hilde Rosing, Jan H.M. Schellens, Jos H. Beijnen. (2010). A new, validated HPLC-MS/MS method for the simultaneous determination of the anti-cancer agent capecitabine and its metabolites: 5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine, 5-fluorouracil and 5-fluorodihydrouracil, in human plasma. http://www.ncbi.nlm.nih.gov/pubmed/19650151

22) N. Sreekanth, Bahlul Z. Awen, Babu Rao ; A new validated RP-HPLC method for the estimation of Capecitabine in bulk and pharmaceutical dosage form http://www.researchgate.net/publication/251442317AnewvalidatedRP-HPLCmethod for the estimation of Capecitabine in bulk and pharmaceutical dosage forms

23) Kumar, K. Ravi; Prasada Rao, CH. M. M.; Rao, CH. Babu; Chandra sekhar, K. B.; Reddy, P. GangiJanuary 2010 RP-HPLC Method development and validation for estimation of capecitabine capsules. http://connection.ebscohost.com/c/articles/51973456/rp-hplc-method-development validation-estimation-capecitabine-capsules

24) R.Svobaite, I.Solassol, F.Pinguet, T.Mazzard, M.Ychou A new, validated HPLC-MS/MS method for the simultaneous determination of the anti-cancer agent capecitabine and its metabolites: 5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine, 5-fluorouracil and 5-fluorodihydrouracil, in human plasma http://www.tandfonline.com/doi/abs/10.1080/10826076.2010.503842#.UnDYCVMlgk

25) Karnaker Reddy, T.; Sravan Kumar, S.; Ravindra Reddy, Y.January 2011Estimation of capcetabine drug present in tablets by RP-HPLC method http://connection.ebscohost.com/c/articles/74080369/estimation-capcetabine-drug- present-tablets-by-rp-hplc-method

26) Christoph Siethoff, Matthias Orth, Andrea Ortling, Erich Brendel[2], Winfried Wagner-Redeker Simultaneous determination of capecitabine and its metabolite 5-fluorouracil by column switching and liquid chromatographic/tandem mass spectrometry http://www.ncbi.nlm.nih. gov/pubmed/15329840

27) Dhananjeyan MR, Liu J, Bykowski C, Trendel JA, Sarver JG, Ando H, Erhardt PW Rapid and simultaneous determination of capecitabine and its metabolites in mouse plasma, mouse serum, and in rabbit bile by high-performance liquid chromatography http://www.ncbi. nlm.nih.gov/pubmed/17070825

28) Rajesh, V.; anupama, B.; jagathi, V.; sai praveen, P. Simultaneous Estimation of Gemcitabine Hydrochloride and Capecitabine Hydrochloride in Combined Tablet Dosage Form by RP-HPLC Method http://www.hindawi.com/journals/jchem/2011/437157/abs/

29) Zufía L, Aldaz A, Giráldez J Simple determination of capecitabine and its metabolites by liquid chromatography with ultraviolet detection in a single injection

http://www.ncbi.nlm.nih.gov/pubmed/15282093

30) Sylvie M. Guichard, Iain Mayer, Duncan I. Jodrell Simultaneous determination of capecitabine and its metabolites by HPLC and mass spectrometry for preclinical and clinical studieshttp://www.ncbi.nlm.nih.gov/pubmed/16198157

31) Salvador, L. Millerioux, A. Renou Simultaneous LC-MS-MS Analysis of Capecitabine and its Metabolites (5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine, 5-fluorouracil) After Off-Line SPE from Human Plasma

http://link.springer.com/article/10.1365%2Fs10337-006-0799-5

32) Yan Xu, Jean L Grem Liquid chromatography–mass spectrometry method for the analysis of the anti-cancer agent capecitabine and its nucleoside metabolites in human plasma

33) http://www.curehunter.com/m/pubmed12450548.do

34) Damien Montange, Michel Bérard, Martin Demarchi, Patrice Muret, Sarah Piédoux, Jean Pierre Kantelip, Bernard Roye An APCI LC-MS/MS method for routine determination of capecitabine and its metabolites in human plasma http://www.ncbi.nlm. nih.gov/pubmed/20527036

35) http://www.xeloda.com/about/isi/

36) http://www.drugbank.ca/drugs/DB01101