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FORCED DEGRADATION STUDIES FOR METHOD DEVELOPMENT AND VALIDATION OF CAPACETABINE BY RP-HPLC

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Abstract

A novel stability indicating liquid chromatographic assay method was developed and validated as per ICH guide lines for the quantitative estimation of Capecitabine in tablet formulation. An isocratic reverse phase LC-method was developed using Hypersil ODS C18, 150× 4.6mm, 5µm column and a mobile phase comprising of a mixture of Ph 2.16± 0.1ortho phosphoric acid and methanol (40:60v/v).The UV-detector set at 242nm with flow rate of 1ml/min. The method is linear between 30µg ml to 70µg ml with R2 value as 0.999, the limit of detection (LOD) is 0.03µg ml and limit of quantification (LOQ) IS 0.09µg ml. The accuracy of the method was found to be in the range of 99.86% to 100.98%.The method precision % RSD were less than 2.Stress degradation studies were done for acid, base ,H2O2 and heat. The proposed method was found to be linear, precise and accurate for the quantitative estimation of Capecitabine in Tablet and can be used for commercial purposes.

Keywords: Capecitabine, HPLC, Stress Stability Indicating and Method validation.

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INTRODUCTION

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Capecitabine is a prodrug, that is enzymatically converted to fluorouracil (antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue.

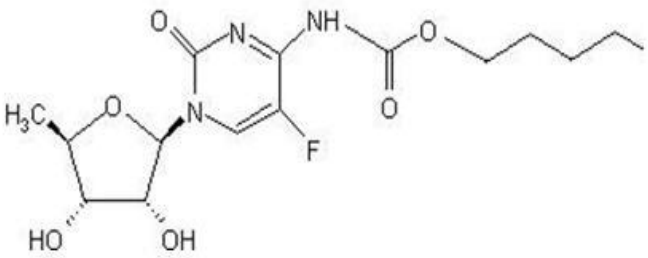

Fig.1: Structure of Capecitabine [1]

HIGH – PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplest and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. To gain an appreciation for the growth of HPLC in pharmaceutical analysis one has only to review the last five years of the journal of pharmaceutical sciences or journal of chromatography. It is common to gain more than 20 papers on analysis of drugs by HPLC in 2003.The rapid growth of HPLC has been facilitated by the development of reliable, moderately priced instrumentation and efficient columns. Separation efficiencies achievable today are 50 times greater than those available in1970s. When 20thedition of USP was issued in 1980, only 20-25 monographs contained in HPLC analysis. Now, HPLC will be one of the most widely used instrumental methods for purity assays by the times USP published in 1999.

HPLC separation is based on interaction and differential partition of the sample between the mobile liquid phase and the stationary phase. The commonly used chromatographic methods can be roughly divided into the following groups, not necessarily in order of importance.

1. Chiral
2. Ion-exchange
3. Ion-pair/affinity
4. Normal phase
5. Reversed phase

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6. Size exclusion

The test method commonly submitted to Center for Drug Evaluation & Research (CDER) is the reverse phase HPLC method. UV detection is the most common detection technique. Reversed phase chromatography, a bonded phase chromatographic technique, uses water as the base solvent. Separation based on solvent strength and selectivity also may be affected by column temperature and pH. In general, the more polar components elute faster than the less polar components. UV detection can be used with all chromatographic techniques. The concern for this type of detector is the loss of sensitivity with lamp aging, and varying sensitivity at the low level depending on design and/or manufacturer. A point to note is that observations on the HPLC chromatograms, by UV detection in combination with reversed-phase HPLC, may not be a true indication of the facts for the following reasons.

Compounds much more polar than the compound of interest may be masked (elute together) in the solvent front/void volume.

Compounds very less polar than the analyte may elute either late during the chromatographic run or retained in the column.

Compounds with lower UV extinction co-efficient or different wavelength maxim may not be detectable at the low level relative to the visibility to the analyte since only one wavelength is normally monitored.

MATERIALS AND METHODS

Instruments Used

Hitachi L-2130 series consisting pump L-2130,Autosampler,UV-Detector L-2400 Hitachi, Thermostat column compartment connected with Hitachi Elite Lachrome (D-2000Elite) software. [2,3]

METHODS

Mobile Phase Selection

On The Basicof Literature Survey, previous Experience and several exploratory efforts. the chromatographic compatibility was achieved by using p^H 2.2 ortho phosphoric acid) and Methanol in a ratio of (40:60)as an Isocratic elution. This gives the results as a mobile phase.

Wavelength selection

After screening the standard solution over 200 to 400 nm wavelength. On the basis of absorption maxima of Analyte 242 nm was decided as maximum wavelength.

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Column selection

Column selection is the most important part in the method development. Applying various column Chemistry. The most suitable selected column was Hypersil ODS $C_{18,}$150×4.6mm.5µ.

Preparation of solutions:

Preparation of Standard solution:

Working concentration should be around 40 µg/ml.

Accurately weighed around 25mg of Capacetabine working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml.

Further dilutions has been made to get the final concentration of 40 µg/ml

Preparation of Test solution:

Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range [4,5].

Chromatographic conditions:

Column	:	C_{18}, 250 X 4.6 mm, 5µ,
Flow rate	:	1 mL /min
Wavelength	:	242nm
Column temperature	:	20°C
Injection volume	:	10 µL
Run time	:	10 minutes
Diluent	:	Mobile phase
Elution	:	Isocratic
Needle wash	:	Ortho phosphoric acid and Methanol 40:60 (v/v)
Mobile phase -	:	orthophosphoric acid and Methanol(40:60)
RT	:	2.96.

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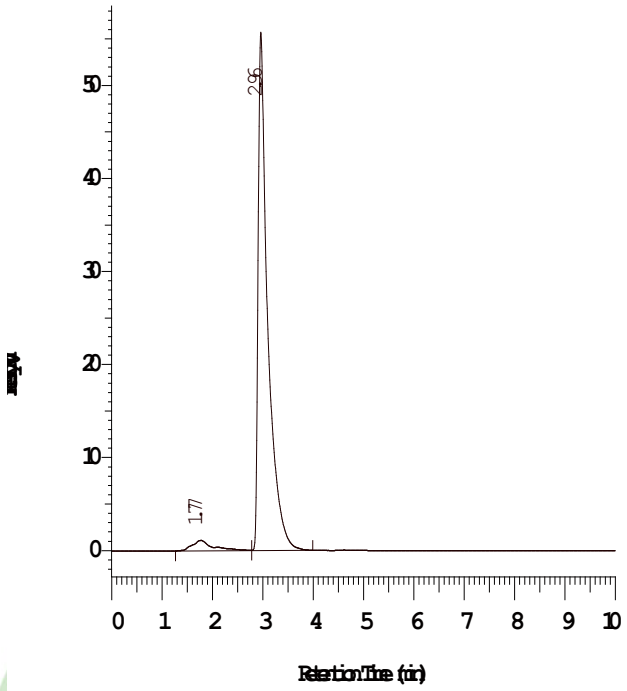

Fig.2: Chromatogram of ortho phosphoric acid and methanol (40:60)

Preparation of sample solutions

Preparation of sample stock solution: Label claim: 50mg

Prepare working standard of 40ppm solution from stored 1000ppm 1N NaOH solution with drug dissolved in it by taking 1ml of it into 10ml volumetric flask and adding diluents to it upto mark which gives 100ppm solution and again repeating same procedure by taking 1ml of 100ppm solution to finally prepare 10ppm solution.

Inject blank 1N NaOH into HPLC and run it.

Then inject 10 ppm solution prepared in 1st step into HPLC and run it.

RESULTS & DISCUSSION

Method development

Table 1: Preparation at 60% to 140% Level:

Linear solutions (%)	Stock solution taken in (mL)	Diluted to volume (mL) with diluents
60%	24	100
80%	32	100
100%	40	100
120%	48	100
140%	56	100

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Table 2: Linearity data of Capecitabine

Analyte	Concentration (µL)	Peak area	%RSD	Linear regression Equation
Drotaverine.Hcl	0	0	0	Y=66757X+27685. R^2=0.9986
	24	4023128	200	
	32	4993646	400	
	40	6929130	500	
	48	3119928	600	
	56	3775190	700	

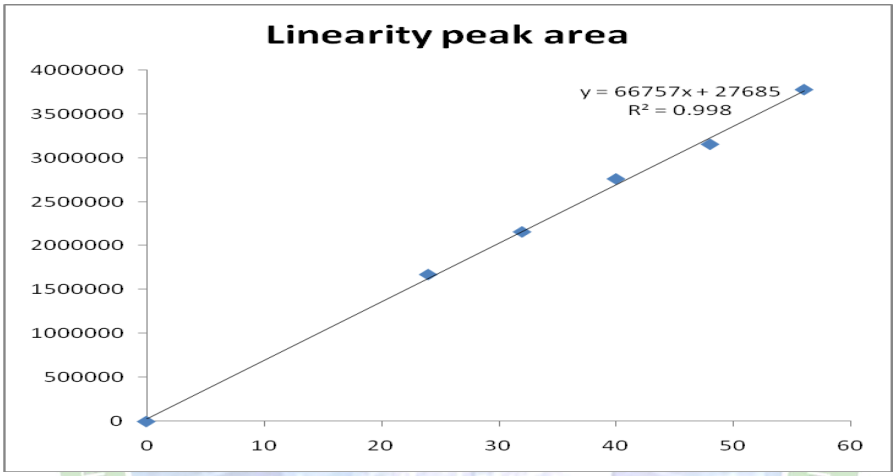

Fig. 3: Calibration curve for Capecitabine

Accuracy/Recovery

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts(80%, 100%, and 120%) of pure drug of Capecitabine were taken and added to the pre-analysed formulation of concentration 30µg/ml from the all above data it has been proven that the % recovery is within the limit of 98 to 102% this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2%. Hence this suggests that proposed method is highly accurate. The average percent recoveries obtained as 98% - 102%. Indicating that the method was accurate.

Table 3

Drug Name	RT	Peak Area	Tailing Factor	Plate count
CAPACETABINE	2.51	748053	2.04	1924

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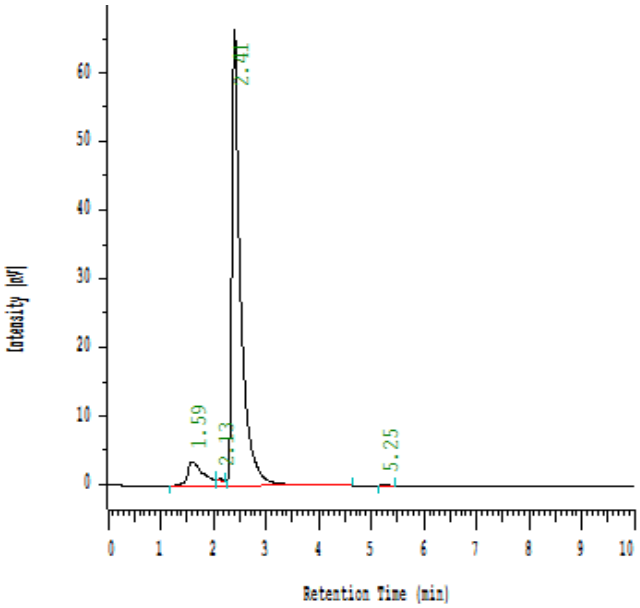

Fig. 4: Accracy of Capecitabine

STABILITY OF CAPACETABINE IN THERMAL CONDITION

Prepare a working standard of 40 ppm from the stock solution and keep in oven at 60℃ for atleast 6 hours to determine its stability. After 6 hours inject the solution and run it.

Conclusion

As therecovery results are fou and between 98.0% to 102%, the study proves that the method is accurate for the estimation.

Method precision: (Repetability)

Precision was demonstrated by prepared six sample preparation as per the test method representing a single batch.

Acceptance criteria:

PRECISION

Preparation of precision solution

10ml of standard stock solution was diluted in 100ml s.v.f up to volume with diluents Repeat the same procedure for remaining six preparations.

% RSD to be tabulated with obtained readings in tabular form.

Acceptance Criteria

The RSD of areas from six preparations precision Level should not be more than 2%.

METHOD PRECISION (Repeatability)

Precision was demonstrated by prepared six sample preparations as per the test method representing a single batch.

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Acceptance Criteria

The RSD of areas from six preparations precision Level should not be more than 2%.

Reproducibility

The intra & inter day precision variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD ≤ 2%) within a day & day today variations for Capecitabine revealed the proposed method is precision.

LOD (Limit of Detection)

Limit of detection is the lowest concentration of analyte in a sample that can be detected .but not necessarily quantities. under the stated experimental condition.

LOQ (Limit of Quantification)

Limit of detection is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Robustness

Influence of small change in chromatographic conditions such as change in flow rate (±0.1ml/min), Temperature (±c^0), wavelength of detection (±2nm)& acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table%RSD≤%) The developed RP-HPLC method for the analysis of Capecitabine (API).flow Rate: The solution was prepared and Injected with (±0.1) flow rate i.e,,0.9ml/min and 1.1ml/min respectively [5,6].

Table 4: Summary of Validation Parameters

S.No	Parameters	Limits	Observed Values
1	System suitability	%RSD shouid be NMT 2	0.18
2	Linearity	Correlation coefficient (r^2)NLT 0.99	0.998&0.999
3	LOD	3mg/ml	0.1
4	LOQ	10mg/ml	0.3
5	Accuracy	% Recovery range is 98-102%	98-100%
6	Intraday Interday % Precision	%RSD should be NMT 2	0.87&0.24
7	Ruggedness	%RSD should be NMT 2	0.33&0.65
8	Robustness	%RSD should be NMT 2	0.24&1.56

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CONCLUSION

The Chromatographic conditions were optimized to develop new method for Capecitabine in API and tablets dosage forms. The basic Chromatographic conditions were designed to be simple and easy to used and reproduced and were selected after testing the different conditions that affect HPLC analysis. Thus New Method has developed which is precise and validated. The developed method was found to be specific and validated as for ICH guide lines.

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REFERENCES

1. https://www.drugbank.ca/drugs/DB01101.
2. Validation of Analytical Procedures, Methodology, ICH Harmonized Tripartite Guidelines, 1996.
3. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
4. Validation of analytical procedures, Methodology, ICH harmonized tripartite guideline, 108, 1996.
5. International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," *Federal Register* 60 (40), 11260–11262 (1995).
6. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," *Federal Register* 62(96), 27463–27467 (1997).