ISSN 0975- 2633

Research Article Arch Pharm Sci& Res Vol 7 No 1 No 1- 1-7 June 2015

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR CAPECITABINE IN FORMULATION AND BULK DRUG

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In the present work, simple and sensitive spectrophotometric methods and RP-HPLC method has been developed for the quantitative estimation of Capecitabine in bulk and in Pharmaceutical formulations.

A rapid and sensitive RP-HPLC Method with UV detection (240nm) for routine analysis of Capecitabine in Bulk and in Pharmaceutical formulation was developed. Chromatography was performed with mobile phase containing a mixture of Trifluoro Acetic Acid and Acetonitrile (35:65 v/v) with flow rate 1ml $min^{-1}$. In the range of 5-30 µg/ml, the linearity of Capecitabine shows a correlation co-efficient of 0.998. The proposed method was validated by determining sensitivity, accuracy, precision, linearity, selectivity and system suitability parameters.

**Key words:** Capecitabine,*RP- HPLC.*

## INTRODUCTION

Capecitabine is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Capecitabine is classified as an "antimetabolite." It is Pentyl[1-(3,4-dihydroxy-5-methyltetrahydrofuran-2-yl)-5-fluoro-2-oxo-1*H*-pyrimidin-4yl]carbamate (fig.1).In literature survey several HPLC methods (Vainchtein LD, 2010, Mugunthu R,2007,Licea-Perez H, 2009,Montange D,2010,Svobaitė R, 2010), are available for estimation in human and rabbit plasma, and for pharmaceutical formulations( Pani Kumar AD et al, 2011, NarendraDevanaboyina et al, 2013, P. Ravisankar et al, 2013, Subhashini.edla, et al, 2012, KarnakerReddy.Y et al, 2011, Sreekanth.N et al, 2010, Medikondu Kishore et al, 2011) are available.

## METHODS AND MATERIALS

Capecitabine (99.8% purity) was received as gift sample from Government Drug Testing Laboratory, Bengaluru, Karnataka. HPLC grade Acetonitrile, Trifluoro Acetic acid and Triethylamine (Merck, Mumbai, India). Pharmaceutical formulation XELODA tablet (label claim 150 mg Capecitabine) was used in HPLC analysis. HPLC grade water obtained in-house by Direct-Q water purification system (Millipore, Milford, USA) used in the estimation of Capecitabine by HPLC.

### Chromatographic Conditionsand Apparatus:

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on a Waters X-bridge-C18 column 5µm 4.6x250mm with UV detection at 240nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data. The mobile phase used was Trifluoro acetic acid pH 2.5 and Acetonitrile (35:65).

### Instrument Parameters

Isocratic flow rate of mobile phase was maintained at 1ml/min, the column temperature was maintained at $24^0$C. The injection volume as 20µl, eluted sample was monitored at 240nm and the run time was 10 min and the retention time of sample was 5.643 min.

### Standard solutions for HPLC estimation Capecitabine

Accurately weighed 50 mg of Capecitabine in 50 ml volumetric flask and dissolved in mobile phase and the volume were made up to the mark with the same solvent. From the above 10 mL solution were pipetted out into separate 100 ml volumetric flask and volume was made up to the mark with the same solvent. This gave the concentration of 100 µg $mL^{-1}$ of Capecitabine, from this six dilutions were prepared and five dilutions in between 5-30 µg $mL^{-1}$ of Capecitabine with mobile phase.

### Sample preparation in HPLC estimation of Capecitabine

Twenty tablets each containing 150 mg of Capecitabine were weighed and powdered for further study.The powder equivalent to 150 mg

of Capecitabine were accurately weighed and transferred to 100mL volumetric flask containing 50mL of the mobile phase and sonicated for 10min. The above solution was carefully filtered through Nylon membrane 0.22µ and the residue was washed with 3 portions of 10 mL of mobile phase. The volume was made up to themark with mobile phase. From this solution, required dilutions for HPLC method were prepared within the linearity range using mobile phase as solvent.

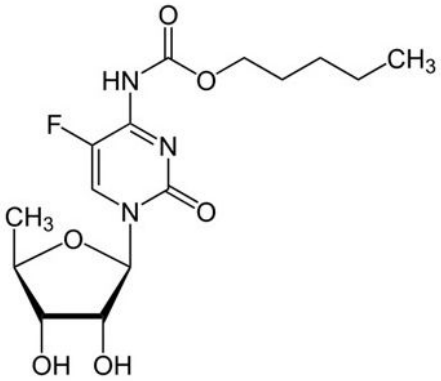


Fig.1 Structure of Capecitabine

**Chromatography**

After several trials with the different combination and ratio of solvents, the mobile phase Acetonitrile :Trifluoro aceticacid (65:35 v/v, pH 2.5±0.1) was selected, because it was found that it ideally resolve the peaks with retention time ($R_t$) 5.643 min and the same is shown in Fig.4. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component shown reasonably good response at 240nm (Fig.2).

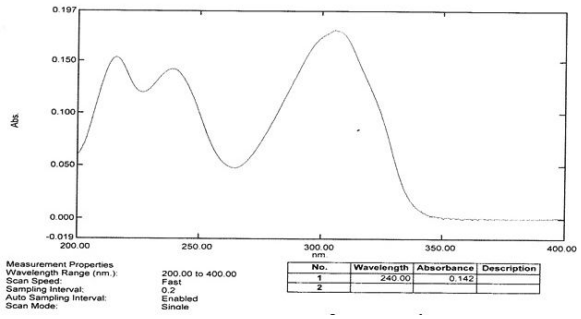


Fig.2.UV-Spectrum of Capecitabine at 240

**Validation of Method**

**Blank interference:**

A study to establish the interference of blank was conducted fig.3. Mobile phase was injected as per the test method. Chromatogram of blank should not show any peak at the retention time of analyte peak.

**Calibration:**

Study was carried out individually for the ingredient at six different concentration levels. All stock and working solutions were sonicated for 10 min then filtered through the nylon membrane filter (0.22µ) prior to use. Triplicate of 20µL injections were made for each concentration and chromatographed under specified condition at ambient temperature ($24^0$C).

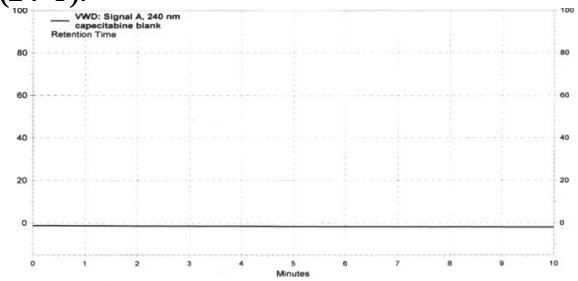


Fig.3. Chromatogram of blank

**Linearity:**

Linearity of the proposed HPLC method for determination of Capecitabine was evaluated by analyzing a series of different concentrations of standard drug. In this study six concentrations were chosen ranging between 5-30µg $mL^{-1}$ for Capecitabine fig.6. Each concentration was injected three times and obtained information on variation in the peak area response of pure analyte was plotted against corresponding concentrations and result was shown in Table 1. The linearity of the calibration graph was validated by the high value of correlation coefficient, slope and the intercept value was shown in Fig.5. The optimized method parameters are given in the Table 5.3.

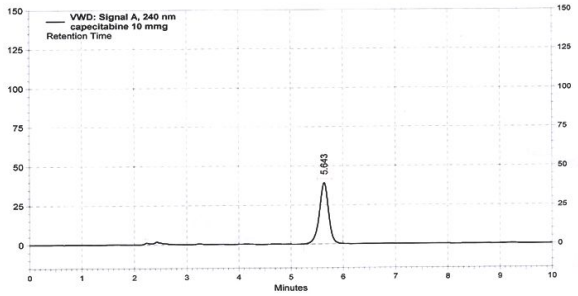

Fig.4. Chromatogram showing Retension time ($_{Rt}$) at 5.63 min for Capecitabine

**Precision**

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. It was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for the title ingredient. The repeatability (within-day in triplicates) fig 7&8 and intermediate precision (for 3 days) fig. 9&10 were carried out at six concentration levels for compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The precision expressed as % RSD.

| Validation Parameters | Capecitabine |
|---|---|
| Mobile phase | Acetinitrile: Trifluoro Acetic acid(65:35 v/v) |
| Flow rate | 1ml/min |
| Detection Wavelength | 240 |
| Rt | 5.63 min |
| Run Time | 10min |
| Theoretical Plates | 4862 per meter |
| LOD | 0.31µg/ml |
| LOQ | 1.05µg/ml |
| Linearity | 5-30 µg/ml |
| Precision | % RSD < 2 |

| S.NO | Concentration (µg/ml) | Peak Area* |
|---|---|---|
| | | Capecitabine |
| 1. | 5 | 5141279 |
| 2 | 10 | 8087577 |
| 3. | 15 | 12137972 |
| 4. | 20 | 15609800 |
| 5. | 25 | 19866705 |
| 6. | 30 | 23576989 |

Table 1.Linearity data for Capecitabine

**Accuracy**

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Capecitabine using standards at 80%, 100% and 120% fig.11,12 &13.Each sample was analysed in triplicate for each level. From the results, % recovery is calculated.

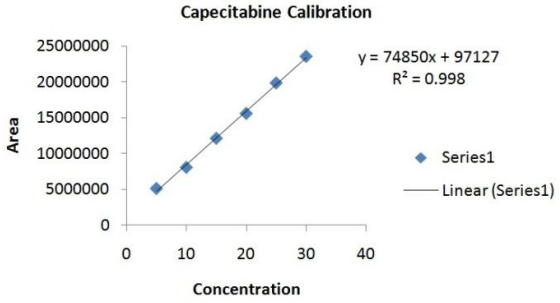


Fig 5.Linearity curve for Capecitabine at 240nm
*Average of six determinations

**LOD and LOQ**

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

**RESULTS**

The objective of the proposed work was to develop a method for the determination of Capecitabine to validate the methods according to USP and ICH guidelines and applying the same for its estimation in laboratory prepared mixtures. HPLC methods developed was found to be rapid, simple, precise, accurate and economic for routine estimation of Capecitabine in laboratory prepared mixtures.

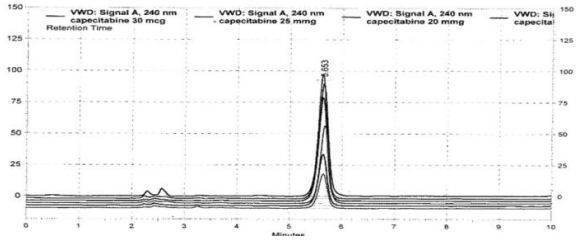


Fig 6.Overlay chromatograms of linearity

In HPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compound. Initially, various mobile phase compositions were tried,

to separate title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, and resolution. The mobile phase, Acetonitrile: Trifluoro Acetic acid (pH 2.5) (65:35 v/v) with 1ml.min$^{-1}$ flow rate is quite robust.

The optimum wavelength for detection was 240 nm at which better detector response for the title drug was obtained. The retention time for Capecitabine was found to be 5.64 min respectively. The calibration was linear in concentration range of (5-30)µg mL$^{-1}$ withregression 0.9998, intercept 971277 and slope 748501 for Capecitabine. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of (98.04-101.24)%.

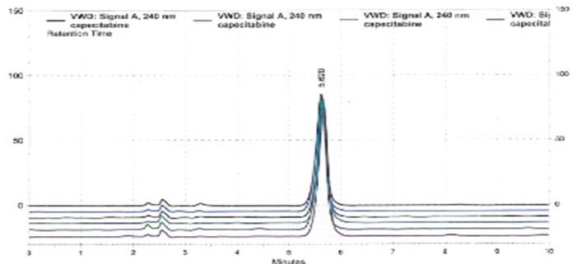

Fig 7.Overlay chromatograms of repeatability (morning)

Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days over a period of three days. These results show the accuracy and reproducibility of the assay. The % R.S.D. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in laboratory prepared mixtures.

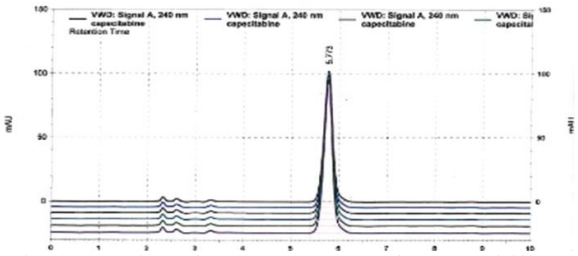

Fig 8.Overlay chromatograms of repeatability (afternoon)

The proposed methods are accurate, simple, rapid and selective for the estimation of Capecitabine in laboratory prepared mixtures. Hence, these methods can be conveniently adopted for the routine analysis of Capecitabine in quality control laboratories.

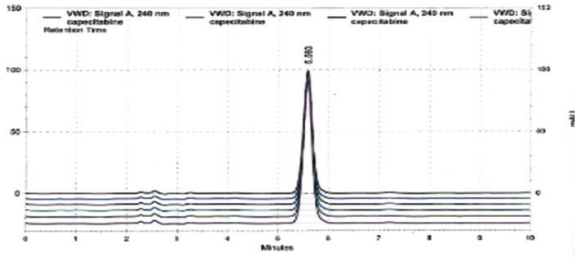

Fig 9.Overlay chromatograms of repeatability (day 1)

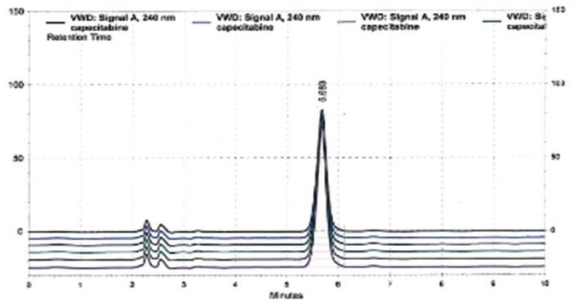

Fig 10.Overlay chromatograms of repeatability (day 2)

| S. No | Label claim in mg | Amount Added in mg | Amount found in mg | %Recovery |
|---|---|---|---|---|
| | Capecitabine | Capecitabine | Capecitabine | Capecitabine |
| 1 | 150 | 75 | 147.06 | 98.04 |
| 2 | 150 | 150 | 152.13 | 101.24 |
| 3 | 150 | 225 | 147.58 | 98.39 |

Table 5.Recovery studies of Capecitabine

| Morning | | | Afternoon | | |
|---|---|---|---|---|---|
| S.No | Conc(25µg/ml) | Area | S.No | Conc(25µg/ml) | Area |
| 1. | Injection 1 | 19715424 | 1. | Injection 1 | 19682549 |
| 2. | Injection 2 | 19316166 | 2. | Injection 2 | 19553162 |
| 3. | Injection 3 | 19253773 | 3. | Injection 3 | 19357112 |
| 4. | Injection 4 | 19219662 | 4. | Injection 4 | 19403877 |
| 5. | Injection 5 | 19545499 | 5. | Injection 5 | 19298313 |
| 6. | Injection 6 | 19261322 | 6. | Injection 6 | 19601921 |
| | Std. dev | % RSD | | Std. dev | %RSD |
| | 199765.3 | 1.030 | | 151724.8 | 0.778762 |

Table 2.Precision (Repeatability) study results of prepared sample

| Day 1 | | | Day 2 | | |
|---|---|---|---|---|---|
| S.No | Conc(µg/ml) | Area | S.No | Conc(µg/ml) | Area |
| 1. | Injection 1 | 19622370 | 1. | Injection 1 | 19585370 |
| 2. | Injection 2 | 19531456 | 2. | Injection 2 | 19676391 |
| 3. | Injection 3 | 19653210 | 3. | Injection 3 | 19431765 |
| 4. | Injection 4 | 19410457 | 4. | Injection 4 | 19327799 |
| 5. | Injection 5 | 1933754 | 5. | Injection 5 | 19597353 |
| 6. | Injection 6 | 19478998 | 6. | Injection 6 | 19667432 |
| | Std. dev | % RSD | | Std. dev | %RSD |
| | 121732 | 0.6240 | | 138982.3 | 0.71099 |

Table 3.Precision (Intermediate Precision) study results of prepared sample

| S.No. | Level in % | Area Response | Mean % recovery |
|---|---|---|---|
| 1 | 50 | 19482822 | 98.04 |
| 2 | | 19476802 | |
| 3 | | 19481716 | |
| 4 | 100 | 19505673 | 101.24 |
| 5 | | 19514712 | |
| 6 | | 19512604 | |
| 7 | 150 | 19551417 | 98.39 |
| 8 | | 19547686 | |
| 9 | | 19542109 | |

Table 4.Assay results of Capecitabine

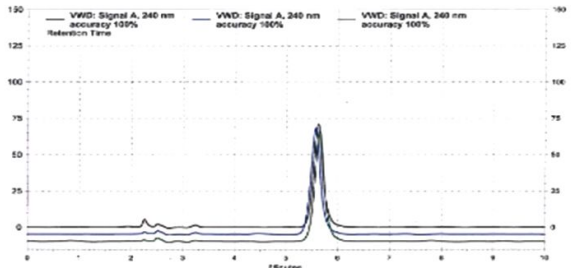

Fig.12. Chromatograms of 100%recovery study

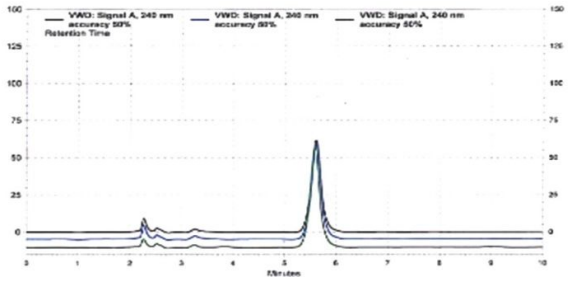

Fig.11. Chromatograms of 50%recovery study

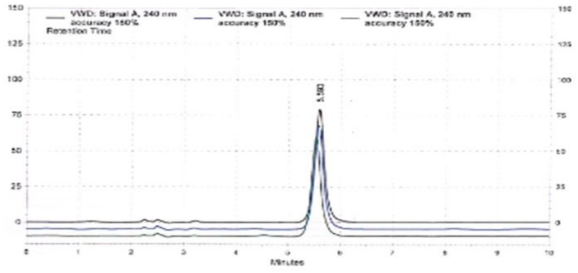

Fig.13 Chromatograms of 150%recovery study

| Parameters | Capecitabine |
|---|---|
| LOD(µg/ml) | 0.31 |
| LOQ(µg/ml) | 1.05 |

Table 6.LOD and LOQ

## SUMMARY AND CONCLUSION

On the basis of the experiments, we can conclude that the RP-HPLC & method developed for the Estimation of Capecitabine can be used for routine analysis Q.C. Samples. Capecitabine was determined by reverse phase HPLC using Acetonitrile (pH 8.0): Trifluoro Acetic acid (65:35v/v) as mobile phase, and Waters X Bridge C 18Column, 5µ 250×4.6mm as a stationary phase. Detection was carried out using UV detector at 240 nm. After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision, and accuracy.

| Validation Parameters | Capecitabine |
|---|---|
| Mobile phase | Acetinitrile: Trifluoro Acetic acid(65:35 v/v) |
| Flow rate | 1ml/min |
| Detection Wavelength | 240 |
| Rt | 5.63 min |
| Run Time | 10min |
| Theoretical Plates | 4862 per meter |
| LOD | 0.31µg/ml |
| LOQ | 1.05µg/ml |
| Linearity | 5-30 µg/ml |
| Precision | % RSD < 2 |

Table 7.Summary of the present study (RP-HPLC)

- The system suitability was found to be within the limits. The limit was Not more than RSD <2. The retention time of Capecitabine is 5.63 mins. The data regarding the system suitability is shown in table 5.1..
- The Specificity of Capecitabine is shown in Chromatogram there was no interference. In this method it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods.
- The precision was found to be within the limits. The limit were not more than RSD <2. This indicates that the method is precise. The data regarding the precision are shown in table 5.4 and 5.5.
- From the linearity table 5.2 it was found that, the drug obeys Beer's Law. For HPLC the calibration plot of Imatinib was observed as linear in the range 5-30µg/ml and the correlation coefficients were found to be 0.998 respectively.
- From the results shown in the accuracy table 5.6 and 5.7, it was found that Recovery value of pure drug from the solution were between 98.04% to 101.24 %. This indicates that the method is accurate.

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