VALIDATED ESTIMATION OF CAPECITABINE BY UV-SPECTROSCOPIC, RP-HPLC AND HPTLC METHOD

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ABSTRACT
This paper describes the Quantitative estimation of Capecitabine in pure powder and its pharmaceutical dosage form by UV spectroscopy (geometric correction method), RP – HPLC and HPTLC methods. In the UV method, geometrical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. In this method, three wavelengths 263, 281, and 293 nm were selected for the estimation of Capecitabine in Serum. Based on the absorbance the corrected absorbance was calculated. Capecitabine was quantified by using the corrected absorbance. In the RP-HPLC method, the drug was resolved using a mobile phase [methanol: buffer (1 ml glacial acetic acid in 1000 ml water) (45:55 % v/v)] on C18 column [develosil ODS-MG-5 (100 nm × 4.6 mm) 5µ] in isocratic mode. The retention time of Capecitabine was found to be 2.17 min. In HPTLC method, the chromatograms were developed by using a mobile phase [Chloroform: glacial acetic acid: methanol (8:1:1 % v/v)] on precoated plate of silica gel 60F254 and quantified by CAMAG TLC scanner 3, at 240 nm. The Rf value of Capecitabine was 0.271. Mean Recovery is 98.8-101.4%, percentage relative standard deviation (%RSD less than 2%) and correlation coefficient (linearity range) that developed methods were accurate and precise. These methods can be employed for the routine analysis of tablets containing Capecitabine.

Key words: Capecitabine, UV spectroscopy, Geometric correction, RP-HPLC, HPTLC Method, validation.

INTRODUCTION
Capecitabine ([deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine, Xeloda], is a fluoropyrimidine carbamate, which is converted in liver and tumour to the active agent 5-fluorouracil (5-FU). It is used in the chemotherapeutic treatment of patients with breast and colon cancer. Capecitabine is a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. The activation of Capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR), to form 5-fluorouracil. Extensive pharmacokinetic studies have been performed on Capecitabine and its metabolites based on phases II and III trials.

A very few physico-chemical methods appeared in the literature for the assay of Capecitabine in biological fluids and pharmaceutical formulations. Most of them are based on HPLC and LC-UV methods, LC-MS/MS, LS-MS/MS, MB methods for its determination in human plasma and pharmaceutical dosage forms. Hence the present work aims to develop accurate, precise, specific, linear, simple and rapid method for the estimation of Capecitabine in pure, pharmaceuticals and in biological sample by UV-spectroscopic, RP-HPLC, and HPTLC methods.

MATERIALS AND METHODS

Reagents & Chemicals
The chemicals used for the study were fresh serum, methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), chloroform (Analytical grade), glacial acetic acid (analytical grade) and methanol (analytical grade).

Instruments
A UV-visible spectrophotometer (1700 shimadzu) with spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 10 mm matched quartz cells photo multiplier tube detector were used. RP-HPLC was performed using Water TM 486- Tunable absorbance detector equipped with water 510 HPLC Pumps, a 717 auto sampler and UV detector. The column was symmetry C18 (develosil ODS-MG-5 (100 nm × 4.6 mm) 5µ used. The method was conducted using an isocratic technique. Data acquisition and processing was performed using Empower software. HPTLC was performed in Camag HPTLC system, equipped with Linomat IV with Camag100 µL Syringe as sample applicator Lamac Twin trough Glass Chamber (20x10) as development chamber, Lamac TLC Scanner III with Wincat software.

UV-METHOD
Preparation of standard stock solution and Selection of wavelengths
Accurately weighed 10 mg of Capecitabine raw material and was transferred in to 10ml volumetric flask and dissolved in water and made up to the volume with water. This solution contains 1 mg/ml concentration. The standard stock solution was further diluted with water to get the concentration of 10 µg/ml and the solution was scanned between 200 to 400 nm using water as blank. From the spectra, Capecitabine showed maximum absorbance at 240nm. The stock solution with biological sample is prepared by taking 10 mg of Capecitabine raw material and transferred in to 10 ml volumetric flask and dissolved in 10 ml of serum. This solution contains 1 mg/ml concentration. The standard stock solution was further diluted with water to get the concentration of 10 µg/ml and the solution was scanned between 200 to 400 nm using same solution excluding drug as blank. From the spectra, Capecitabine showed maximum absorbance at 281 nm. Three wavelengths (263, 281, and 293) nm were selected for Geometric correction method by observing the overlain spectrum of Capecitabine with and without serum.

Preparation of standard stock solution [without serum]
Accurately weighed 10 mg of Capecitabine raw material and was transferred into 10ml volumetric flask and dissolved in water and made up to the volume with water. This solution contains 1 mg/ml concentration.
Linearity and calibration graph [without serum]
The standard stock solution containing 1000 µg/ml was further diluted to get the concentration of 60 µg/ml of Capecitabine. From the working stock solution of Capecitabine (0.5-2.5 ml) was transferred into series of five 10 ml volumetric flasks and made up to the volume with water. The absorbance of different concentration solutions were measured at their selected wavelengths.

Preparation of standard stock solution [with serum]
Accurately weighed quantity of 10 mg of Capecitabine raw material was added in to the 10 ml of serum and sonicate for 10 min. This solution contains 1 mg/ml concentration.

Linearity and calibration graph [with serum]
The standard stock solution containing 1000 µg/ml was further diluted with water to get the concentration of 60 µg/ml of Capecitabine. From the working stock solution of Capecitabine (0.5-2.5 ml) was transferred into series of five 10 ml volumetric flasks and made up to the volume with water. The absorbance of different concentration solutions were measured at their selected wavelengths. For the Geometric correction method, corrected absorbances were found by using the formula.

Corrected absorbance, \( D = \frac{\lambda_1 - \lambda_2}{\lambda_1 - \lambda_3} \)

Quantification with serum
Twenty tablets of Capecitabine (Xeloda) were accurately weighed and powdered. The powdered tablet equivalent to 10 mg of Capecitabine was weighed and added in to the 10 ml of serum and sonicated for 10 mins. From the working stock solution 2.5 ml was taken in a 25 ml volumetric flask and made up to volume with water to get 100 µg/ml concentration.

The solution was further diluted with water, to give concentration of 10 µg/ml of Capecitabine, and filtered through whatman filter paper no.41. Absorbance of these solutions was measured six times at their selected wavelengths (263 nm, 281 nm, 293 nm) using blank. Blank was prepared same as sample preparation excluding drug.

Quantification without serum
The above same procedure was repeated without serum sample. The amount of Capecitabine present in formulation was calculated by using corrected absorbance from the slope and intercept of respective calibration curve.

RP- HPLC METHOD
The buffer was prepared by adding 1 ml glacial acetic acid in 1000 ml water. The drug was resolved using a mobile phase of methanol:buffer (45:55 % v/v) , filtered using membrane filter and degassed. The flow rate was 1.0 ml/min and the effluent were monitored at 245 nm.

A stock solution was prepared by dissolving 10 mg of Capecitabine in 10 ml of mobile phase. The stock solution was further diluted with the mobile phase to obtain final concentration range of (10–60 µg/ml). These solutions were used to calculate the linearity and the relative quantification of the tablets. About 20 tablets were weighed and powdered. A powdered tablet equivalent to 10 mg was weighed accurately and transferred to 10 ml volumetric flask. The tablet powder was dissolved in the mobile phase and filtered through a membrane filter under vacuum filtration. The sample was suitably diluted to get the concentration of 20 µg/ml and injected, under the specified conditions and the chromatogram was recorded. The amount of Capecitabine present in tablet was determined by using slope and intercept values from the calibration graph.

HPTLC METHOD
The drug was resolved using a mobile phase chloroform: glacial acetic acid: methanol (8:1:1 % v/v). Chamber and plate saturation time was 30 min, migration distance was 50 mm and UV detection was carried out 240 nm. A stock solution was prepared by dissolving 10 mg of Capecitabine in 100 ml of methanol (1000 µg/ml). The stock solution was further diluted with methanol to obtain final concentration range (100-600 ng/µl). The standard solutions were applied on the TLC plate. The chromatograms were developed. The developed chromatograms were evaluated by scanning in densitometric mode at 240 nm. The calibration curve was constructed by using peak area against concentration. The procedure was repeated for six times. The sample solution was prepared as that of HPLC method and filtered through whatman filter paper. Finally the sample was diluted to get a concentration (300 ng/µl). The 1µl of sample was applied on the TLC plate. The chromatogram was developed. The developed chromatograms were evaluated by scanning in densitometric mode at 240 nm. The content of Capecitabine in tablet was calculated by using slope and intercept values from the calibration graph.

LOD and LOQ
The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD & LOQ were calculated as

\[ \text{LOD} = 3.3\sigma / S \quad \text{LOQ} = 10\sigma / S \]

Where, \( \sigma \) is the standard deviation of the lowest standard concentration and \( S \) is the slope of standard curve.

RECOVERY STUDIES
Recovery studies were carried out by adding known quantities of standard at different levels to the pre analyzed sample to study the linearity, accuracy and precision of the proposed method. The recovery studies also reveals whether there is positive or negative influence on the quantification parameters by the additive usually present on dosage forms.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Capcitabine</th>
<th>Standard Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>2.717</td>
<td></td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.39</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Asymmetrical factor</td>
<td>1.39</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>4642051</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Theoretical plate per unit length</td>
<td>4143</td>
<td>-</td>
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</table>
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Table 2: Summary of Validation Parameters for Capecitabine by the Proposed UV, RP-HPLC and HPTLC

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>UV</th>
<th>HPLC</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limit</td>
<td>3 – 15 (μg/ml)</td>
<td>10 – 60 (μg/ml)</td>
<td>100 – 600 (ng/ml)</td>
</tr>
<tr>
<td>Correlation coefficient(r²)</td>
<td>0.9991</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.393458x + 11.88846</td>
<td>y = 120526.27x + 3237324.044</td>
<td>y = 1634.966 x + 5.142889</td>
</tr>
<tr>
<td>Slope</td>
<td>0.393458</td>
<td>120526.27</td>
<td>1634.966</td>
</tr>
<tr>
<td>Intercept</td>
<td>11.393458</td>
<td>3237324.044</td>
<td>5.142889</td>
</tr>
<tr>
<td>LOD</td>
<td>0.0264 (μg/ml)</td>
<td>0.1294 (μg/ml)</td>
<td>0.002453 (ng/μl)</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.0802 (μg/ml)</td>
<td>0.3923 (μg/ml)</td>
<td>0.007434 (ng/μl)</td>
</tr>
</tbody>
</table>

Table 3: Recovery analysis for Capecitabine by the Proposed UV, RP-HPLC and HPTLC methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Amount present μg/ml</th>
<th>Amount added μg/ml</th>
<th>Amount found μg/ml</th>
<th>Amount received μg/ml</th>
<th>%Recovery</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>4.91</td>
<td>7.1</td>
<td>12.79</td>
<td>14.91</td>
<td>4.80</td>
<td>99.79</td>
<td>0.4219</td>
</tr>
<tr>
<td></td>
<td>4.91</td>
<td>10.03</td>
<td>16.93</td>
<td>18.99</td>
<td>100.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.91</td>
<td>12.01</td>
<td></td>
<td>20.1</td>
<td>100.05</td>
<td>0.0866</td>
<td>0.0866</td>
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<tr>
<td>RP-HPLC</td>
<td>20</td>
<td>9.82</td>
<td>29</td>
<td>18.99</td>
<td>99.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.9</td>
<td>39.10</td>
<td>20.1</td>
<td>100.05</td>
<td>0.0866</td>
<td>0.0866</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30.09</td>
<td>50.19</td>
<td>20.1</td>
<td>100.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPTLC</td>
<td>334</td>
<td>241</td>
<td>574</td>
<td>333</td>
<td>99.70</td>
<td>0.2886</td>
<td>0.2890</td>
</tr>
<tr>
<td></td>
<td>334</td>
<td>300</td>
<td>635</td>
<td>333</td>
<td>100.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>334</td>
<td>361</td>
<td>694</td>
<td>333</td>
<td>99.70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of six observations

RESULT AND DISCUSSION

In the UV method

Three wavelengths 263, 281, and 293 nm were selected for the estimation of Capecitabine in Serum and in without serum. The spectrum was shown in figure-2. The linearity of Capecitabine was found to be 3-15 μg/ml and r²=0.999. The recovery values were 99.79-100.4 % with percentage relative standard deviation (%RSD) of 0.42.

In RP-HPLC method

The mobile phase was optimized with methanol:buffer (45:55 % v/v) on C18 column in isocratic mode. Sharp peak was obtained with the retention time 2.17 min. The UV detection was carried out at 245 nm. An optimized chromatogram of Capecitabine was shown in figure-3. The System suitability parameters (Table-1) were applied to a representative chromatograph to check various parameters such as with the IP requirements. Linearity range of 10-60 μg/ml and r²=0.9999. The recovery values were 99.9 % to 100.05 % with percentage relative standard deviation (% RSD) of 0.0866.

In HPTLC method

The chromatograms were developed by using a mobile phase chloroform: glacial acetic acid: methanol in the ratio of (8:1:1 % v/v) on precoated plate of silica gel 60F254 and quantified by densitometric absorbance mode at 240 nm. The optimized chromatogram was shown in figure-4. The Rₜ value of 0.271
with a linearity range of 100 to 600 ng/µl and \( r^2 = 0.999 \). The recovery values were 99.44 -101.14% with %RSD of 0.2886. In these three methods the validation parameters results were shown in Table-2. Recovery values were shown in Table-3. All the method validation parameters are well within limits as specified in the ICH guidelines. The validation parameters results were shown in Table-3.

CONCLUSION
The UV, HPLC and HPTLC methods developed for Capecitabine shows good precision and accuracy. The low %RSD values in the recovery studies for these method shows that there is no interference due to excipients used in the formulation. Hence it was concluded that the developed methods are simple, precise, accurate and rapid for the analysis of Capecitabine in pure and in tablet dosage form. Thus the developed methods can be adopted for the routine analysis of Capecitabine in bulk and tablet dosage form.

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