Development and Validation of Spectrofluorimetric Method for the Quantification of Capecitabine in Bulk and Tablets

Short title: Spectrofluorimetric Quantification of Capecitabine

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ABSTRACT
Objectives: A new, simple and affordable spectrofluorimetric method was established for the quantification of capecitabine in bulk and in marketed formulation.

Materials and methods: Native fluorescence of capecitabine in 0.1% w/v cetrimide was measured at 386 nm after excitation at 313 nm.

Results: Linear relationship between fluorescence intensity and the capecitabine concentration was noticed in 0.2-1.0 µg/mL range. The method was supported by checking several validation parameters as stated by ICH guidelines. The limit of detection and quantification values (0.032 and 0.096 µg/mL, respectively) and results of validation parameters demonstrated that the method procedure was sensitive, accurate, precise and reproducible (% relative standard deviation < 2.0). The % assay in commercial formulation was found to be 99.2, which is in agreement with ICH guidelines.

Conclusion: As a consequence of the above findings, developed method can be successfully adopted in routine analysis of capecitabine in pharmaceutical dosage forms.

Key words: Capecitabine, Spectrofluorimetry, Linearity, Accuracy.

INTRODUCTION
Capecitabine is chemically known as pentyl N-[1-[(2R, 3R, 4S, 5R)-3,4-dihydroxy-5-methyloxolan-2-yl]-5-fluoro-2-oxopyrimidin-4-yl] carbamate (Figure 1). Being an antineoplastic drug, it is used in the treatment of breast and colorectal cancers.1,2 Extensive literature review of Capecitabine disclosed several analytical methods for its quantification either alone or in combination with other drugs. Visible spectroscopic method in methanol3, UV spectrophotometric methods in various solvents, such as methanol, ethanol, water, 0.1 N NaOH, 0.1 N HCl, water:acetonitrile (50:50)1-12, electrochemical analysis13, atomic absorption spectroscopic method14, HPLC methods in combinations of stationary and mobile phases15-27, HPTLC method11,28, UPLC method29,30, bioanalytical methods using HPLC31-35, HPTLC36, LC-MS/MS37 and UHPLC-MS/MS method38 were reported in literature.

Although numerous instrumental techniques are available as on date, no spectrofluorimetric method has been reported for capecitabine using cetrimide as solvent to the best of our cognition. Chromatographic methods (HPLC, HPTLC, UPLC, etc) require costly instrumentation, skilled technicians and expensive solvents. Spectrofluorimetry attained exceptional status in the drug analysis because of its appreciable specificity and sensitivity. Unlike spectrophotometry, the analysis can be achieved at both excitation and emission wavelengths in spectrofluorimetry39. Keeping these facts in view, a simple, extraction free and sensitive spectrofluorimetric method was attempted for Capecitabine using cetrimide as solvent. The method was validated as stated in ICH guidelines40 and the same with success utilized for quantification of Capecitabine in marketed dosage form.
MATERIALS AND METHODS

Chemicals
Capecitabine (API) was procured from Gland Pharma, Hyderabad. The marketed formulation containing Capecitabine (Xeloda tablets, Sunrise Remedies Pvt. Ltd., Gujarat) was acquired from a nearby drug store.

Instrumentation
Various instruments like Digital balance (Shimadzu, AUX 220D, Japan), pH Meter (Elico L120, Hyderabad, India), Ultra Sonicator (Sonica Ultrasonic Cleaner, Italy), Melting point apparatus (DBK, Mumbai, India), UV-Visible spectrophotometer (1800, Shimadzu, Japan), FTIR- Spectrophotometer (IR Affinity 1, DRS 8000, Shimadzu, Japan) and Spectrofluorometer (Shimadzu, RF 5301PC, Japan) were used in the present investigation. The standard statistical functions in MS-EXCEL were utilized to compute statistical parameters like arithmetic mean (AM), standard deviation (SD) and percent relative standard deviation (%RSD).

Chemicals and reagents
Cetrimide (0.1% w/v)
Cetrimide was accurately weighed (0.1 g) and dissolved in adequate distilled water (in a volumetric flask) to make 100 mL.

Capecitabine stock solution
A stock containing 1000 μg/mL of Capecitabine was produced by transferring 10 mg of analyte to a 10 mL of 0.1% w/v cetrimide in a volumetric flask and the contents were mixed well. From these aliquots were transferred into 10 mL volumetric flasks and were suitably diluted with 0.1% w/v cetrimide so as to obtain final concentration of 10 and 100 μg/mL of Capecitabine.

Analytical method development
The spectrofluorimetric method development for capecitabine was attempted by dissolving the analyte in various solvents. Cetrimide 0.1% w/v was found to be suitable through optimization studies. The stock solution containing 10 μg/mL of capecitabine in cetrimide 0.1% w/v was used to identify the excitation and emission wavelengths. The excitation wavelength was fixed and solutions were scanned to get the emission spectra. Capecitabine showed fluorescence at emission wavelength 386 nm following excitation at 313 nm, when used 0.1% w/v cetrimide as blank.

Analytical method validation
The emerged method was validated as stated in International Conference on Harmonization (ICH) specifications to prove applicability of the analytical method in quality control of the capecitabine.

Linearity
A set of 10 mL volumetric flasks holding aliquots of Capecitabine in 0.2 - 1.0 μg/mL range in 0.1% w/v cetrimide was prepared. The fluorescence intensities of above solutions were recorded for fluorescence at λ<sub>em</sub> 386 nm using appropriate blank. The features of the calibration curve, such as slope, intercept along with correlation coefficient were computed.

Accuracy (recovery studies)
The degree of closeness of the results was determined by computing recoveries of capecitabine adopting method of standard additions. Standard solutions of capecitabine at 80, 100 and 120% level were spiked to a fixed concentration of capecitabine from the tablet powder (equivalent to 0.5 μg/mL) contained in volumetric flasks. The volume in each of the flasks was made up to mark with 0.1 % w/v cetrimide. The fluorescence intensities of the emerged solutions were resolute at emission wavelength, 386 nm. The recovery was verified by analyzing analyte in triplicate preparations at each concentration level.

Precision
The repeatability/intra-day precision of the present method was set by assessing the corresponding response three times in a single day for three distinct concentrations of capecitabine (0.2, 0.6 and 1.0 μg/mL). The intermediate/inter-day precision was deliberated by estimating selected concentrations (0.2, 0.6 and 1.0 μg/mL) response in triplicate on three different days over a week period. The results of both the studies were expressed as percentage relative standard deviation (%RSD).

Sensitivity and robustness
Sensitivity of the analytical method was represented by determining the lowest detectable amount (LOD) and the lowest quantifiable amount (LOQ) using samples containing very low concentrations of capecitabine as per ICH guidelines. The LOD and LOQ were deliberated using the formulae 3.3*(standard deviation/slope) and 10*(standard deviation/slope), respectively. The fluorescence intensity of the analyte solutions was also recorded by making small changes in the emission wavelength in order to establish robustness in the analytical method.

Assay of capecitabine in pharmaceutical dosage form
Twenty tablets of marketed formulation (Xeloda), containing 500 mg of capecitabine were taken, precisely weighed and powdered. A quantity of powder analogous to 10 mg of capecitabine was transferred into a 10 mL volumetric flask and the volume was made up to mark with 0.1 % w/v cetrimide (1000 μg/mL). The resulting solution was screened via Whatman filter paper (N0. 41). An aliquot of the clear filtrate was suitably diluted to obtain 0.5 μg/mL of capecitabine in 0.1 % w/v cetrimide and the same was utilized for testing. The amount of capecitabine was determined by substituting responses into equations of the straight line representing the calibration curves, with correction for dilution.

RESULTS AND DISCUSSION

Analytical method optimization

Capecitabine structure contains 5-fluoro-pyridimidin-2-one moiety in conjugation with an amide group. The fluorescence potential of capecitabine may be attributed to above mentioned chromophoric groups. The present fluorometric method was optimized by studying the type and concentration of solvents. Stock solutions of Capecitabine in solvents, such as water, methanol, chloroform, dimethylsulfoxide, acetate buffer (pH 5.0), potassium dihydrogen orthophosphate (pH 3.0), Tween 80 (0.25% v/v), sodium lauryl sulphate (0.1 N), urea (0.1 M), cetrimide, sodium hydroxide (0.1 N) and hydrochloric acid (0.1 N) were prepared separately from respective standard solutions (1000 μg/mL). The fluorescence intensity of Capecitabine in cetrimide was found to be maximum among all. Hence, cetrimide was chosen to be a suitable solvent for further analysis. Capecitabine in cetrimide exhibited maximum fluorescence at emission wavelength 386 nm following excitation at 313 nm (Figure 2). Hence the same wavelengths were utilized in further method optimization. The effect of concentration of cetrimide on fluorescence intensity of the analyte was studied by testing different concentrations of cetrimide (0.1, 0.25, 0.5, 0.75 and 0.1% w/v). Sample solutions of Capecitabine (10 μg/mL) prepared by using above cetrimide solutions were scanned and the results were provided in Table 1. Cetrimide, 0.1% w/v was found to be optimum, as maximum fluorescence potential was noticed with the same. Capecitabine sample solutions prepared by dissolving in 0.1% w/v cetrimide were found to be stable up to 48 hours at room temperature.

Analytical method validation

Linearity

The optimized method was further justified as per ICH guidelines. The relationship between capecitabine concentration and corresponding fluorescence intensity was found linear over 0.2-1.0 μg/mL concentration range with a r² of 0.9991. The regression equation obtained was y=9.8571x +0.1514. The method established a good correlation between concentration and fluorescence intensity of Capecitabine over the studied concentration range. The results of linearity studies were given in Table 2 and Figure 3.

Accuracy (recovery studies)

Three distinct levels (80,100 and 120 %) of standards (in triplicate) were spiked to commercial tablet powder to determine accuracy of the proposed method. The mean % recoveries and % RSD of the same were calculated and reported in Table 3. The %recoveries were found to be 96.22, 108.00 and 105.45, respectively for the three levels. The results were found to be satisfactory, which was indicated by the %RSD< 2.0.

Precision

Triplicate samples of three dissimilar concentrations containing 0.2, 0.6 and 1.0 μg/mL of capecitabine were utilized for ascertaining the intra- and inter-day variability. Results of these studies were provided in Table 4. The %RSD values were found to be < 2.0, indicating satisfactory exactness of the method.

Sensitivity and robustness

The sensitivity of the analytical method was evidenced with LOD and LOQ values. They were found to be 0.032 and 0.096 μg/mL, respectively. Further, the robustness of the proposed method was established by evaluating the influence of small variations in the emission wavelength at 386 ± 2 nm. The results indicated that these changes did not greatly affect the fluorescence intensity.

Analysis of Capecitabine in marketed formulation

The contemplated method was applied with success in commercial tablets. The amount of capecitabine in the formulation was found to be 496±2.83 mg and the %assay was 99.2 (Table 5), which is in the acceptance range of 98.0-101.0% for capecitabine as per ICH guidelines. The % RSD less than 2.0 indicated the reliability of the present method. The details of optimized conditions for spectrofluorimetric method of capecitabine were given in Table 6.

CONCLUSIONS

The present spectrofluorimetric method developed for quantification of capecitabine in 0.1 % w/v cetrimide was found to be simple, sensitive and rapid. High scope of the method was evidenced through analysis of validation parameters. This method is more suitable while working with low levels of capecitabine, as the linearity range established over 0.2 – 1.0 μg/mL concentrations confirms the same. Adoptability of the method in the quality control analysis of capecitabine was ascertained in the marketed tablets. Non-interference of the formulation excipients in
the actual determination of capecitabine was noticed. The %assay values and %RSD values obtained during accuracy and precision studies were within the ICH stated limits. With the said features, the contemplated spectrofluorimetric method can be employed for routine quality control analysis of capecitabine in tablet dosage forms.

REFERENCES

Figure 1. Chemical structure for capecitabine
Figure 2. Excitation and emission spectra of Capecitabine in 0.1% w/v cetrimide

Figure 3. Calibration curve of capecitabine (0.2 - 1.0 μg/mL) in 0.1% w/v cetrimide

\[ y = 9.8571x + 0.1514 \]
\[ R^2 = 0.9991 \]
Table 1. Effect of concentrations of cetrimide on capecitabine at $\lambda_{em}$ 386 nm

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (%w/v)</th>
<th>Fluorescence intensity at 386 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>106.627</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>74.588</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>44.781</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>56.643</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>35.618</td>
</tr>
</tbody>
</table>

Table 2. Calibration curve data of capecitabine at $\lambda_{em}$ 386 nm

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/mL)</th>
<th>Fluorescence intensity at 386 nm (AM ± SD) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2.30±0.095</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>4.15±0.253</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>6.01±0.303</td>
</tr>
</tbody>
</table>
Table 3. Data for accuracy studies of capecitabine

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery level %</th>
<th>Conc of sample (µg/mL)</th>
<th>Conc of standard spiked (µg/mL)</th>
<th>Total amount (µg/mL)</th>
<th>Amount recovery (AM±SD) (µg/mL) (n=3)</th>
<th>%Recovery</th>
<th>%RSD(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine</td>
<td>80</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
<td>0.866±0.0035</td>
<td>96.22</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>1.08±0.006</td>
<td>108.00</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.5</td>
<td>0.6</td>
<td>1.1</td>
<td>1.16±0.020</td>
<td>105.45</td>
<td>1.72</td>
</tr>
</tbody>
</table>

AM: Arithmetic Mean, SD: Standard Deviation
\(^a\)Acceptance Criteria: % RSD should not be more than 2.0.

Table 4. Data for precision of analytical method

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration estimated(µg/mL) (AM ± SD)</td>
<td>%RSD(^a)</td>
</tr>
<tr>
<td>0.2</td>
<td>0.23±0.0018</td>
<td>0.78</td>
</tr>
<tr>
<td>0.6</td>
<td>0.727±0.0047</td>
<td>0.65</td>
</tr>
<tr>
<td>1.0</td>
<td>0.946±0.0034</td>
<td>0.36</td>
</tr>
</tbody>
</table>

\(^a\)Acceptance criteria: %RSD should not be more than 2.0
Table 5. Assay of capecitabine in the marketed formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label claim (mg)</th>
<th>Amount found (mg) (AM±SD) (n=3)</th>
<th>% Assay</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeloda®</td>
<td>500</td>
<td>496±2.83</td>
<td>99.2</td>
<td>0.55</td>
</tr>
</tbody>
</table>

AM: Arithmetic mean, SD: Standard deviation
*A: Acceptance criteria: %RSD should not be more than 2.0

Table 6. System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Capecitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation wavelength (nm)</td>
<td>313</td>
</tr>
<tr>
<td>Emission wavelength (nm)</td>
<td>386</td>
</tr>
<tr>
<td>Linearity range (µg/mL)</td>
<td>0.2 - 1.0</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>9.8571</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.1514</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 9.8571x + 0.1514</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9991</td>
</tr>
<tr>
<td>Accuracy (%RSD)</td>
<td>Less than 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Precision (%RSD)</strong></td>
<td>Less than 2.0</td>
</tr>
<tr>
<td><strong>LOD (µg/mL)</strong></td>
<td>0.032</td>
</tr>
<tr>
<td><strong>LOQ (µg/mL)</strong></td>
<td>0.096</td>
</tr>
<tr>
<td><strong>Assay (%)</strong></td>
<td>99.2</td>
</tr>
</tbody>
</table>