Analytical Method Development and Validation of Capecitabine in Bulk Dosage form by UV-Spectrophotometry

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Abstract - A robust UV-visible spectroscopic method was developed and validated for the quantification of Capecitabine in bulk dosage form. The method is based on the drug's ultraviolet absorbance, optimized at a maximum wavelength of 325 nm. Using ethanol as the solvent, the method demonstrated linearity within the concentration range of 5–25 µg/ml, adhering to Beer's law. Validation of the method encompassed parameters such as accuracy, precision, specificity, and linearity, ensuring its reliability for pharmaceutical analysis. Recovery studies further affirmed the accuracy and applicability of the technique for routine quality control. The simplicity, rapidity, and cost-effectiveness of this UV-visible spectroscopic method make it an efficient tool for analyzing Capecitabine in bulk pharmaceutical formulations

Key Words: Capecitabine, Beer's law, Uv spectroscopy

INTRODUCTION

CONCEPT OF METHOD DEVELOPMENT IN UV SPECTROMETRY

Determination of pharmaceutical compound by UV spectroscopy can be done in two ways.

- Qualitative analysis
- Quantitative analysis

The basic criteria for determination of a compound by UV spectroscopy in the compound must have a conjugated double bond in its structure. So that the electronic excitation occurs when it absorbs in UV light at the region of 200-400mm.

Any of the following electronic excitation takes place by the compounds when absorbs UV light. Possible electronic transitions of a л, σ and n electrons are

- $\sigma - \sigma^*$Transitions
- n-σ^*Transitions
- n-$\pi^*$and л- π^*Transitions

The absorption maxima of the unknown compounds can be calculated mathematically by Woodward's fisher rule. The given compound solution should obey beer lamberts law. Method development of the given pharmaceutical compound by UV spectroscopy can be done by Qualitative Analysis

Determining the Effect of Conjugation, Geometric isomerism, Alkyl substitution and No of rings in structural analysis of organic compounds.

Detection of impurities

Structure elucidation of Organic compounds.

1.1 QUANTITATIVE ANALYSIS

Assay of substance in single component samples

Single standard (or) direct comparison method

In this method the absorbance of a standard solution of known concentration and a sample solution is measured. The concentration of unknown can be calculated using the formula,

$$C_2 = C_1 \times \frac{A2}{A1}$$

Were,

A_1 A_2=Aberrance of standard and sample.

C_1 C_2 =Concentration of standard and sample

Calibration curve method or multiple standard methods

A calibration curve is plotted using concentration Vs absorbance value of five (or) more wed solutions. A straight line is drawn either through maximum number of point or in way that there is equal magnitude of positive and negative errors that is line of best fix. From the hence of the sample solution and using the

calibration curve, the concentration of drug mount and the percentage purity can be calculated.

Standard absorptivity value method

In this method, the use of standard A (1%, cm) or values used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the References substances.

Assay of substances in multicomponent samples

In multicomponent samples spectral interference can arise which is known as irrelevant Non-specific absorption, it arises from absorption by other materials and impurities that may be present Spectral selectivity and detection sensitivity can be enhanced significantly by a number chemical or instrumental techniques, which include difference, higher derivative and dual-wavelength spectrophotometry. Such methods and certain graphic techniques such as the Mt Stubbs method, can contribute in different ways to reduce the general problem of spectral interference in quantitative spectroscopy. When interference arises specifically from the spectral overlap of two or more well defined components, a number of methods can be applied to the measure the individual concentrations.

The basis of all the UV-spectrophotometric techniques for multicomponent samples should have the property. At all wavelengths, absorbance of a solution is the sum of absorbance of the individual components present in solution; The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The additional criterion is that the components present in the combined dosage form do not interact chemically and the entire component should soluble in the same solvent.

Simultaneous equation method

The present spectroscopic estimation carried out by Simultaneous equation method (Vie rot's method) If a sample contains two absorbing drugs (X and Y) each of which absorbs at the of the other, it may be possible to determine both drugs by the technique of simultaneous equations provided that certain criteria apply.

The information required is

The absorptivites of X at λ_1, and λ_2, a_{x1}, and a_{x2} respectively

The absorptivites of Y at λ_1 and λ_2, a_{y1} and a_{y2}, respectively

The absorbance's of the diluted sample at A and AA, and A, respectively.

Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1, and λ_2, the absorbance of the mixture is the sum of the individual absorbances of X and Y.

At λ_1 $A_1 = a_{x1}\, bc_x + a_{y1} bc_y.$

At λ_2 $A_2 = a_{x2}\, bc_x + a_{y2} bc_y.$

At λ_1

$A_1 = a_{x1}\, bc_x + a_{y1} bc_y.$

At λ_2

$A_2 = a_{x2}\, bc_x + a_{y2} bc_y.$

Rearrange equation (2)

$C_{Y=}A_2\text{-}a_{x2}c_x/A_{y2}$

Substituting for C_y in eq. (1) and rearranging gives

Cx= A2ay1-a1ay2 / ax2 ay1- ax1 ay2

And

CY= A1 ax2-A2 ax1/ax2 ay1-ax1 ay2

Data required for the construction of simultaneous equation.

The following steps should be followed:

The Amax of the drug should be found out by using the reference standards of drugs.

The calibration of curve should be plotted for each drug and the linearity range should be found out.

The absorbance values of each reference drugs at the two wavelengths (max point of two drugs) should be measured and their absorptivity values should be calculated.

The absorbance values of the tablet formulation at the two wavelengths should be measured and recorded.

Criteria for obtaining maximum precision

The ratio $(A_1/A_2)/ax_2/ax_1$, $(ay_2/ay_1:)/A_2/A_1$, should lie outside the range of 0.1-2.0 for the precise determination of two drugs X and Y.

Theλ_{max} of the X and Y are dissimilar.

The two components should not react chemically.

The additivity of the individual absorbances of the two components should be confirmed to the total absorbances of the sample i.e...

Total Absorbance of the sample = Absorbance of X+ Absorbance of Y. (Beckett A.H and Stenlake J.B., 2001)

Advantages

- Simple and it can be employed for routine analysis of a combination of drugs.
- Very less time is required for analysis when the absorptivity values are determined.
- Only two selected wavelengths are enough for the determination of absorbance of samples.

Area under curve method

In this method, the absorptivity values ($\varepsilon_{1}$and ε_{2}) of each of the two drugs were determined at the selected wavelength range. Total area under curve of a mixture wavelength range. This method is applicable when theλ_{max} of the two components is reasonably dissimilar, the two components do not interact chemically and both the component must be soluble in same solvent.

The methods deviated when overlapping of UV spectra of two drugs significantly and large difference in labelled strength. The accuracy of the method depends upon the nature of solvent, pH of solution, temperature, high electrolyte concentration and the presence of interfering substances.

1.2 Validation of Analytical Parameters as Per ICH Guidelines

The discussions of the validation of analytical procedures are directed to the four most common types of analytical procedures:

Identification Tests

Quantitative tests for impurities' content.

Limit tests for the control of impurities.

Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- Accuracy
- Intermediate Precision
- Specificity
- Detection Limit
- Precision
- Repeatability
- Quantization Limit
- Linearity

SPECIFICITY

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The products used to demonstrate specificity will depend on the analytical procedure. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination) In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

LINEARITY

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity. Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. For the establishment of linearity, a minimum of five concentrations is recommended.

RANGE

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy, and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered.

- For the assay of a drug substance or a finished (drug) product: Normally from
  80 to 120 percent of the test concentration;
- For content uniformity: Covering a minimum of 70 to 130 percent of the test Concentration, unless a wider, more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified;
- If assay and purity are performed together as one test and only a 100 percent Standard is used; linearity should cover the range from the reporting level of the impurities to 120 percent of the assay specification.

ACCURACY

Accuracy should be established across the specified range of the analytical procedure

Assay

Drug substance

Several methods of determining accuracy are available.

(a)Application of an analytical procedure to an analyte of known purity (e.g, reference material)

(b) Compression of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and or defined

(c)Accuracy may be inferred once precision, linearity, and specificity have been established

Drug product

several methods for determining accuracy are available

(a)Application of the analytical procedures in synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added

(b) In cases where it impossible to obtain samples of all drug product components, it may be acceptable either to add knows quantities of the analyte to the drug product or compare the obtained from a second, well characterized procedure, the accuracy of which is stated and /or defined.

(c)Accuracy may be inferred once precision, linearity, and specificity have been established.

Recommended Data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure) Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference been the mean and the accepted the value together with the confidence intervals.

PRECISION

Validation of tests for assay for quantitative determination of impurities includes an investigation of precision.

Repeatability

Repeatability should be assessed using;

- A minimum of 9 determinations covering the specified range for the procedure
  (e.g,3 concentrations /3 replicates each) or
- A minimum of 6 determinations at 100 percent of the test concentration

Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to he used. The applicant should establish the effect of randoms events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to say these effects individually. The use of an experimental design (matrix) is encouraged

Reproducibility

Reproducibility is assessed by means of an interlaboratory trial Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedure in pharmacopoeias.

These data are not part of the marketing authorization dossier.

Recommended limit

The standard deviation, relative standard deviation (coefficient of variation), and confidence interval

should be reported for each type of precision investigated.

DETECTION LIMIT

Several approaches for determining the detection limit are possible, depending on: whether the procedure is non instrumental or instrumental Approaches other than those listed below may be acceptable.

Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be the reliably detected.

Based on the Standard Deviation of the Response and the Slope

The detection unit (D) may be expressed as:

Limit of detection =3.3 $\boldsymbol{\sigma}$/S

Where, σ = the standard deviation of the response

S= the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

QUANTITATION LIMIT

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is non instrumental or instrumental.

Approaches other than those listed below may be acceptable.

Based on visual Evaluation

Visual evaluation may be used for non-instrumental methods, but may also use with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable and precision.

Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

Limit of Quantification = 10 $\boldsymbol{\sigma}$/S

Where σ = the standard deviation of responses

S= the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- Stability of analytical solutions
- Extraction time

SYSTEM SUITABILITY TESTING

System suitability is an integral part of many analytical procedures. That test is based on the concept that the equipment, electronics, analytical operations, and samples to analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

1.3 INSTRUMENTATION

Ultraviolet-Visible Spectroscopy

Absorption of this relatively high-energy light causes electronic excitation. The easily accessible part of this region ranges from 200 to 800 nm. It shows absorption only if conjugated pi-electron systems are present. Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases.

Absorbance is directly proportional to the path length 'b' and the concentration 'c' of the absorbing species.

Principle

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be

absorbed as the electron is promoted to a higher energy orbital.

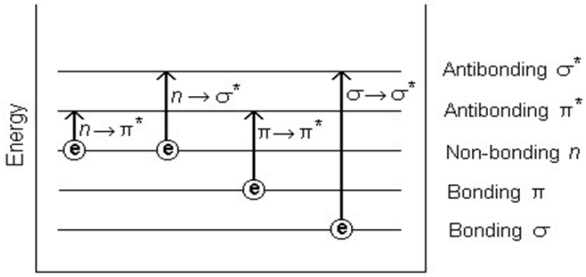


Fig no.1 Electronic Transition Process in a Molecule

As a rule, energetically favoured electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is said to be at excited state.

Electronic transitions

The absorption of UV or visible radiation corresponds to the excitation of outer electrons. There are three types of electronic transition which can be considered;

1. Transitions involving л, σ, and п electrons.
2. Transitions involving charge-transfer electrons
3. Transitions involving *d* and *f* electrons.

When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level.

Absorbing species containing л, σ and n electrons

Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band. Possible electronic transitions of, π σ and n electrons are;

σ-σ* Transitions

An electron in a bonding & orbital is excited to the corresponding antibonding orbital. The energy required is large. For example, methane (which has only C-H bonds, and can only undergoes σ -σ *transitions) shows an absorbance maximum at 125 nm. Absorption maxima due to σ-σ* transitions are not seen in typical UV-Vis. spectra (200 - 700 nm)

n-σ* Transitions

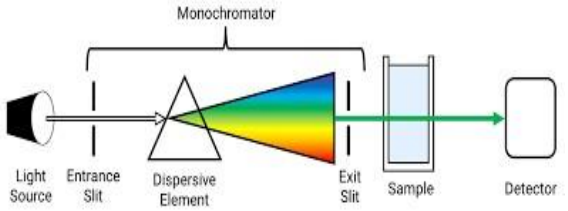
Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of n-σ^* transitions. These transitions usually need less energy than σ -σ* transitions They can be initiated by light whose wavelength is in the range 150 – 250nm. The number of organic functional groups with n-σ^* peaks in the UV region is small.

n-$\boldsymbol{\sigma}^*$ and л-$\boldsymbol{\pi}^*$ Transitions

Absorption spectroscopy of organic compounds is based on transitions of n or electrons to the л* excited state. This is because the absorption peaks for these transitions fall in an n experimentally convenient region of the spectrum (200 - 700 nm). These transitions need an unsaturated group in the molecule to provide the л electrons'.

Fig no 2 Instrumentation

Schematic diagram of a single beam spectrophotometric experiment



Schematic diagram of a double beam spectrophotometer

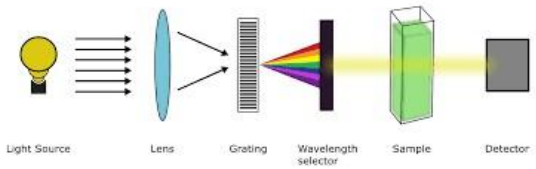


Fig.no.3 Instrumentation of single and double beam spectrophotometer

THE LIGHT SOURCE:

- A deuterium discharge lamp for UV region (160 - 375 nm)
- A tungsten filament lamp or tungsten-halogen lamp for visible and NIR regions (350 -2500 nm)
- Xenon Lamp (190-800 nm)

The instruments automatically swap lamps when scanning between the UV and visible-NIR regions.

THE MONOCHROMATOR

Accepts Polychromatic Input Light from lamp and outputs monochromator Light.

All monochromators Contains the following parts

- An Entrance Slit
- A Collimating Lens
- A dispersive device (usually Prism or a Grating)
- A focusing lens
- An Exit Slit

Dispersive Device

Prism

The prisms disperse the light radiation into individual colours or wavelengths. These are found in inexpensive instruments. The Band Pass is lower than that of filters and hence it has better resolution. The resolution depends upon the size and refractive index of the prism. The material of the prism is normally glass. It is of two types Refractive and Reflective.

Grating:

Gratings are the most efficient ones in converting a polychromatic to monochromatic light in the real sense. As a resolution of ± 0.1nm could be achieved by using gratings are of two types Diffraction and Transmission gratings.

THE CUVETTE (OR CELL)

These are containers for the sample and reference solutions and must be transparent to the radiation passing through. UV Region; Quarts or Fused silica Cuvettes

(Also transparent in the VIS/NIR Regions)

VIS\NIR Regions: Silicate glass or Plastic Cuvettes (350-2000 nm).

LIGHT DETECTORS

Photon Transducers: Light→ Electric current

Photomultiplier tube

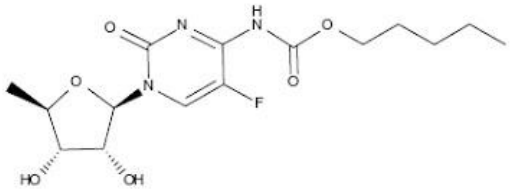
Photoconductivity transducers

Photovoltaic cells

Silicon photodiodes

DRUG PROFILE OF CAPECITABINE

Structure:



IUPAC Name: pentyl N-(1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-methyloxolan-2yl]-5-fluoro-2- oxo-1,2-dihydropyrimidin-4-yl) carbamate

Chemical Formula: C_{15} H_{22} FN_3 O_6

Average Weight: 359.3501

CAS number: 154361-50-9

Type: small molecule

State: solid

Melting point: 110-121 "C

logP: 0.4

Water solubility: 26 mg/ml.

Description:

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.

Pharmacokinetics

Absorption:

T_{max} is approximately 1.5 h for capecitabine and 2 h for 5-FU. Food decreased C_{max}, 60% and AUC 35% for capecitabine and decreased C_{max}. 4.3% and AUC 21% for 5-FU. Food delayed T_{max} 1.5h.

Distribution:

Less than 60% protein bound (approximately 35% bound to albumin).

Metabolism:

Enzymatically metabolized to 5-FU (active) in tissues; also metabolized to inactive metabolites in the liver.

Elimination:

The t is approximately 0.75 h (for capecitabine and 5-FU). Approximately 95.5% is excreted in urine (57%

of the dose as inactive metabolite and 3% of the dose as unchanged drug); 2.6% is excreted in feces.
Absorption:
Readily absorbed through the GI tract (~70%)
Protein binding:
< 60% (mainly albumin)
Volume of distribution:
Not Available
Storage/ Stability:
Store at controlled room temperature (59° to 86°F).

2.3. AIM AND OBJECTIVE

Literature survey reveals that certain chromatographic methods were reported for quantitative estimation of capecitabine and no method is available for the estimation of capecitabine by spectrometry. Information regarding its physical and chemical properties, various analytical methods that were reported either individually or in combination with other drugs. Hence there is a need for a suitable spectrometry method for routine analysis of capecitabine in formulation. To validate the proposed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the capecitabine in dosage form.

2.4. PLAN OF WORK

Literature reveals different methods for their analysis in their formulation. But our present plan is to develop a new, simple, precise and accurate stability indicating spectrometric method for its analysis in formation and to study the effect of buffers on the drug.
STEP-1: Study of physicochemical properties of the drug.
(p^H, p^{Ka}, solubility and molecular weight)
STEP-2: Selection of maximum wave length of the drug.
STEP-3: Optimization of the method.
STEP-4: Study of the system suitability parameters.
STEP-5: Validation of the proposed method.

3.EXPERIMENTAL WORK

3.1MATERIALS AND METHODS

3.1.1. Equipment /Instrument details

S.no	Instrument Name	Model
1	UV system	Shimadzu
2	Semi micro balance	Sartorius ME235P
3	pH Meter	Thermo electron coration orion 2 star
4	Sonicator	Ultrasonic cleaner power sonic 420
5	Vacuum oven	Wadegati
6	Constant temperature water bath	Thermolab GMP

Table no:1: List of Equipment / Instrument details

3.1.2 Chemicals and Reagents

SNO	NAME	MANUFACTURER	GRADE
1	Capecitabine Working standard	Cipla PHARMACEUTICALS	
2	Capecitabine tablets	Ranbaxy pvt. LTD	
3	Ethanol	Milli-pore	Milli-Q
4	0.45mm PVD filter	Rankem	D004A07

Table no.2: list of Chemicals and Reagents

3.2. ANALYTICAL METHOD DEVELOPMENT

A. Selection of wavelength

A solution of 100µg/mL of Capecitabine was prepared in Ethanol. The resulting solutions were scanned individually in UV-Visible spectrophotometer from 190 to 400 nm. The optimal response for the drug was obtained at 325 nm. Hence the complete method was processed at the wavelength of 325 nm. The solvent selected to dissolve the drug was ethanol because of its favourable UV transmittance, low viscosity and free solubility.

Analytical method development and validation of Capecitabine in bulk dosage forms by UV.
Preparation of standard solution: 25 mg of Capecitabine working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask add about 70ml of ethanol was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. (Stock solution) Further 10ml of Capecitabine was pippeted from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:
10 Tablets of Capecitabine were weighed and powdered in glass mortar. The powder equivalent to the amount of active ingredient present in 10 tablets

(156.8mg) was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 1.0ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 um filter.
Diluent Preparation: Ethanol was used as Diluent.

Test Procedure:
10 µg/ml of the Standard, Sample were taken and scanned over the range of 190-400nm.
The scanned UV spectrum for capecitabine is shown in Fig.No.4

3.3. METHOD VALIDATION
The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are:
1. Specificity
2. Linearity
3. Accuracy
4. Precision
5. Ruggedness
6. Robustness

1. SPECIFICITY
A) Capecitabine identification:
Solutions of Standard and Sample were prepared as per test procedure and absorbance values are noted at maximum wavelength of the drug.
Acceptance criteria
Absorbance value of standard and sample should be identical with near value.

2. LINEARITY
Preparation of stock solution:
25 mg of Capecitabine working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and about 70ml of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. (Stock solution)
Preparation of Level - I (5ppm of Capecitabine):
0.5ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with Ethanpl.
Preparation of Level - II (10ppm of Capecitabine):
1.0ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with diluent.
Preparation of Level-III (15ppm of Capecitabine):
1.5ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with diluent.
Preparation of Level - IV (20ppm of Capecitabine):
2.0ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with diluent.
Preparation of Level - V (25ppm of Capecitabine)
2.5ml of stock solution was taken in 10ml of volumetric flask diluted up to the mark with diluent.
Procedure:
Each level solution was scanned in UV spectrophotometer at 325nm and absorbance was measured. A graph of peak area versus concentration (on Xaxis concentration and on Y-axis Absorbance) was plotted and the correlation coefficient was calculated.
The linearity of the method was demonstrated over the concentration range of 5-25 µg/ ml. A calibration curve was plotted for concentration v/s peak area and is given in the Fig.No.5
The results are discussed in Table no.3
Acceptance criteria
1) Correlation Coefficient should be not less than 0.9990.
2)% RSD of peak areas for Solutions of 5-25 ug/ml should be not more than 2.0%

3. ACCURACY
Assay was performed in triplicate for various concentrations of Capecitabine equivalent to 50, 100, and 150% of the standard amount was scanned in UV spectrophotometer at 325mm and absorbance was measured.

Preparation of Standard stock solution:
25 mg of Capecitabine working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and about 70ml of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent.

Preparation Sample solutions:
For preparation of 50% solution (With respect to target Assay concentration):
50 mg of Capecitabine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask about 7ml of Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. (Stock Solution). Further 0.2ml of Capecitabine of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):
100 mg of Capecitabine working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask about 7ml of Diluent was added and sonicated dissolve it completely and volume was made up to the mark with the same solvent (Stock solution). Further 0.2ml of Capecitabine of the above stock solutions was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):
15.0mg of Capecitabine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask, about 7ml of Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent (Stock solution). Further 0.2ml of Capecitabine of the above stock solution was pipetted into a 10ml volumetric flask and dilute up to the mark with diluent

Procedure:
Standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were scanned in UV Amount found and Amount added for Capecitabine individual recovery and mean recovery values were also calculated. The average % recovery of Capecitabine were calculated and the results are summarized in Table no.4.

Acceptance criteria
The mean % recovery of the Capecitabine at each spike level should be not less than 98.0% and not more than 102.0 %.

4. PRECISION
a) Repeatability:
Preparation of stock solution:
25 mg of Capecitabine working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask about 70ml of Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. (Stock solution). Further 1.5ml of Capecitabine of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. (15ppm)

Procedure:
The standard solution was scanned in UV for five times and the absorbance was measured for all five injections. The %RSD for the area of five replicate injections was found to be within the specified limits.
The UV spectrums are presented as The results are given in Table no.5.
Fig.No.9.

Acceptance criteria
1. All individual assays of Capecitabine tablets should be within 98 % - 102 %.
2. Relative standard deviation of % Assay results should not be more than 2.0

b) Intermediate precision (Analyst to Analyst variability): To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by using different columns of same dimensions.

Preparation of stock solution: 25 mg of Capecitabine working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask about 70ml of Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent (Stock solution). Further 1.5ml of Capecitabine of the above stock solution was pipetted out in to a into a 10ml volumetric flask and diluted up to the mark with diluent.
Procedure:
The standard solution was scanned for five times and the area for all five solutions was measured. The %RSD for the area of five replicate injections was found to be within the specified limits.
Two analysts as per test method conducted the study. For Analyst-1 Refer Precision (Repeatability) results

and the results for Analyst-2 are discussed in Table no.6.
UV spectrum is shown in Fig.No.10.

Acceptance criteria
1. All the individual assays of Capecitabine tablets should be within98%- 102 %.
2. Relative standard deviation of % assay results should not more than 2.0% by both the analysts.

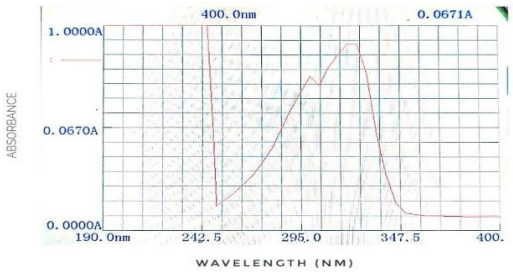
5. RUGGEDNESS
This was performed by different analysts on different days. The UV spectrum for Day-1, Analyst-1 is presented in Fig.No9. and the results are illustrated in Table no.5. The UV spectrum for Day-2, Analyst-2 is given in Fig.No10. and the results are discussed in Table no.6.

Acceptance criteria
1. The % assay for Capecitabine tablets should be between 98.0% - 102 %.
2. The RSD of % assay Capecitabine tablets from the six sample preparations should be no more than 2.0 %.

6. ROBUSTNESS
The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like composition, temperature variations which may differ but the responses were still within the specified limits of the assay.

4.1. RESULTS AND DISCUSSION
The present report in this thesis is aimed at new analytical method development for the estimation of Capecitabine by UV method. From the literature review it was found that there was no single method for the estimation of Capecitabine by UV method. Hence, new analytical method has been developed for the estimation of Capecitabine by UV method and validated according to ICH Q2B guidelines.

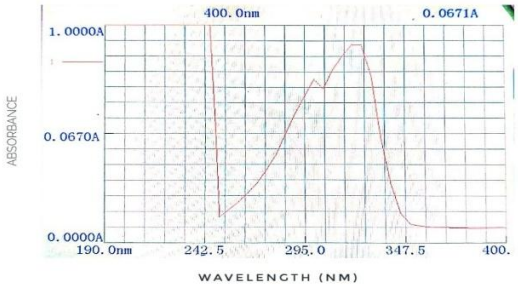
4.1. ANALYTICAL METHOD DEVELOPMENT
A solution of 100ug/ml of Capecitabine was prepared in distilled water.
The resulting solutions were scanned individually from 190 to 400 nm in UV Visible spectrophotometer.
The optimal response was obtained at 325 nm. Hence, the complete method
was processed at the wavelength of 325nm. The spectrum is shown in Fig.No.4.



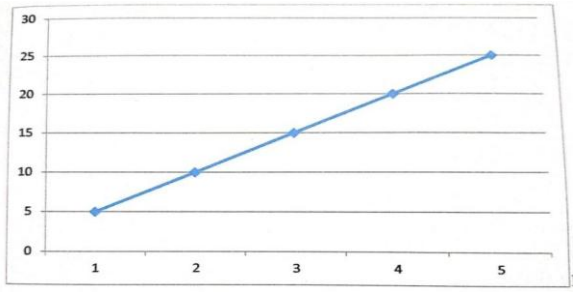
UV spectrum of capecitabine in distilled water

4.1.3. METHOD VALIDATION
1.SPECIFICITY
THE absorbance value of standard and sample are identical with nearly same values. Hence, the given drug passes specificity.

2. LINEARITY
From the Linearity data it was observed that the method was showing linearity in the concentration range of 5-25 ug/ml. Correlation coefficient was found to be 0.999. The data of linearity is illustrated in Table no.3. The spectrums for the linearity are presented and the linearity curve is plotted and given in Fig.No.5.



UV spectrum of capecitabine(5mg) in distilled water at 325nm



Calibration curve of Capecitabine

S.NO	Linearity level	Concentration	Absorbance
1	1	5ppm	0.269
2	2	10ppm	0.582
3	3	15ppm	0.657
4	4	20ppm	1.177
5	5	25ppm	1.312

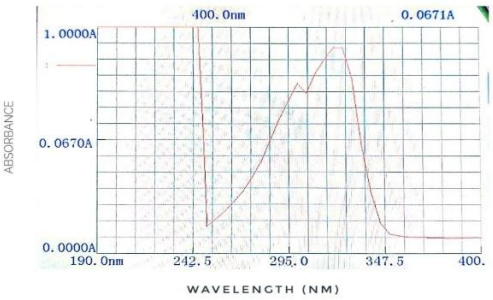
Correlation Coefficient 0.997

Table no.3: Linearity results for Capecitabine

Results: correlation coefficient of capecitabine was found to be 0.995[NMT 0.999]

3.ACCURACY

The recoveries of pure drug from the analysed solution of formulation were 99.22 % to 100.11%, which shows that the method was accurate. The summary of Accuracy results were expressed in Table no.4. And spectrums for accuracy were shown in Fig.No.6.-8



Spectrum for Accuracy50% Conc

Sample No.	Spike Level	Amount (mg /ml) added	Amount (mg/ml) found	%Recovery	Mean % Recovery
1	50%	5	4.96	99.2%	
	50%	5	4.99	99.8%	100.3 %
	50%	5	5.1	102%	
2	100%	10	9.92	99.2%	
	100%	10	9.94	99.4%	99.4%
	100%	10	9.98	99.8%	
3	150%	15.3	15.1	98.6%	
	150%	15.3	15.2	99.3%	99.3%
	150%	15.3	15.3	100%	

Table no.4: %Recovery Results for Capecitabine

Results:

The % Recovery for 100% Accuracy level of Capecitabine was found to be 99.4% (98.0 to 102.0%)

4. PRECISION:

The RSD of % Recovery for Capecitabine of repeatability precision was found to be 0.42 % and 0.86% and in intermediate precision it was found to be 0.42% and 0.86 %. It passes repeatability and intermediate precision. The results of precision were summarized in Table no.5. The spectrums related were represented as Fig. No. 9.

A) Repeatability:

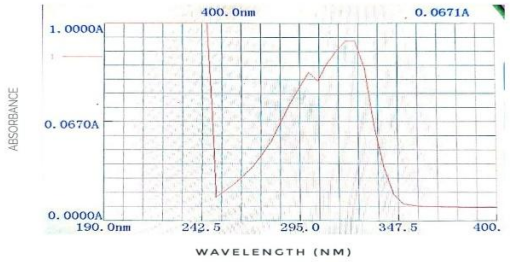


Fig 9. a; Sample spectrum for Repeatability (15ppm)

Injection No	Absorbance	%Recovery
1	0.657	99.4%
2	0.632	100%
3	0.590	99.0%
4	0.576	99.8%
5	0.590	99.2%
MEAN	0.577	99.48%
SD	0.402	0.415
%RSD	0.42	0.42

Table no:5: Sample spectrum value for Repeatability of Capecitabine

Result: The % RSD for the area of five spectra results of Capecitabine was found to be 0.42. (NMT 2).

Fig10.a; Spectrum for intermediate precision

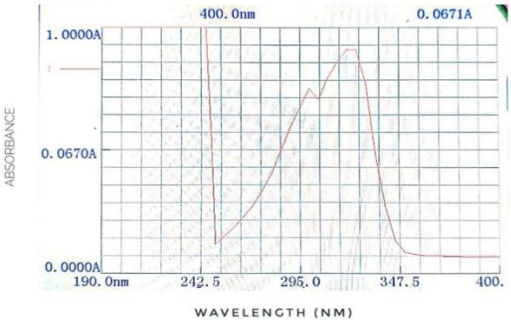


Fig10. e; Spectrum for intermediate precision

Injections no	Absorbance	%Recovery
1	0.596	101.8%
2	0.597	99.8%
3	0.631	99.1%
4	0.598	99.7%
5	0.632	101.2%
Avg	0.598	100.2%
SD	0.52	1.13
%RSD	0.42	0.03

Table no.6: Sample spectrum value for intermediate precision of capecitabine

Result: The % RSD for the area of five standard spectrum results of Capecitabine was found to be 0.03. (NMT 2)

4.2.1 CONCLUSION

For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. UV method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

Capecitabine was freely soluble in ethanol, methanol, acetonitrile and in distilled water. Ethanol was chosen as the mobile phase The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit; hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-50 µg/ml. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate separately and analysis being performed by different analysts.

Good agreement was seen in the assay results of pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Capecitabine in Bulk drug and pharmaceutical formulation.

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