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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CAPECITABINE IN BULK DOSAGE FORM BY UV SPECTROPHOTOMETRY

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ABSTRACT

A simple, rapid-accurate UV method was developed for the determination of Capecitabine in pure and tablet dosage forms. The objective of this validation of an analytical procedure is to demonstrate that the drug Capecitabine is suitable for its intended purpose. The method is based on the ultraviolet absorbance maximum of the above drug at 239.5 nm (240nm). The drug obeyed Beer's law in the concentration range of 5-25µg/ml in distilled water. The proposed method was successfully applied for the determination of drug in commercial tablet preparations. The results of the analysis have been validated statistically and by recovery studies. Due to simplicity, rapidity and accuracy of the method, we believe that the method willbe useful for routine quality control analysis.

KEYWORDS: Capecitabine, Beer's law.

INTRODUCTION

Concept of Method Development In UV Spectrometry

Determination of pharmaceutical compound by UV spectroscopy can bedone 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 electronsare

- $\sigma - \sigma^*$ Transitions
- n-σ^* Transitions
- n-π^* 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 unknowncan 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 negativeerrors 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 isknown 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.

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 thatcertain criteria apply.
The information required is

The absorptivites of X at $\lambda 1$, and $\lambda 2$, a_{x1}, and a_{x2} respectivelyThe absorptivites of Y at $\lambda 1$ and $\lambda 2$, 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.

Area under curve method
In this method, the absorptivity values ($\varepsilon 1$and $\varepsilon 2$) 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.

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)

$$C_Y = A_2 - a_{x2} c_x / A_{y2}$$

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

$$C_x = A_2 a_{y1} - a_1 a_{y2} \,/\, a_{x2}\ a_{y1} - a_{x1}\ a_{y2}$$

And

$$C_Y = A_1\, a_{x2} - A_2\, a_{x1} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$$

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 validationof 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 procedureis 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.

Identification
Suitable identification tests should be able to

discriminate between compounds of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied tomaterials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sensible scientific judgment with a consideration of the interferences that could occur.

Assay and Impurity Test(s)

For chromatographic procedures, representative chromatograms shouldbe used to demonstrate specificity, and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques. Critical separations in chromatography should be investigated at an appropriate level. For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other. In cases where a nonspecific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used.

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. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. 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.

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 analyticalprocedure

Assay

Drug substance

Several methods of determining accuracy are available.

1. Application of an analytical procedure to an analyte of known purity (e.g, reference material)
2. 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
3. Accuracy may be inferred once precision, linearity, and specificity have beenestablished

Drug product

Several methods for determining accuracy are available

1. 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
2. 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.
3. Accuracy may be inferred once precision, linearity, and specificity have beenestablished.

Precision

Validation of tests for assay for quantitative determination of impurities includesan 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 dependson 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 trialReproducibility 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 precisioninvestigated.

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 alsobe 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 σ/S
Where, σ = the standard deviation of the responseS= 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 the Standard Deviation of the Response and the Slope
The quantitation limit (QL) may be expressed as:

Limit of Quantification = 10 σ/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 shouldshow 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 consequenceof 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.

System Suitability Testing
System suitability is an integral part of many analytical procedures .That test are 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.

1.2 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 thatmatches 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.

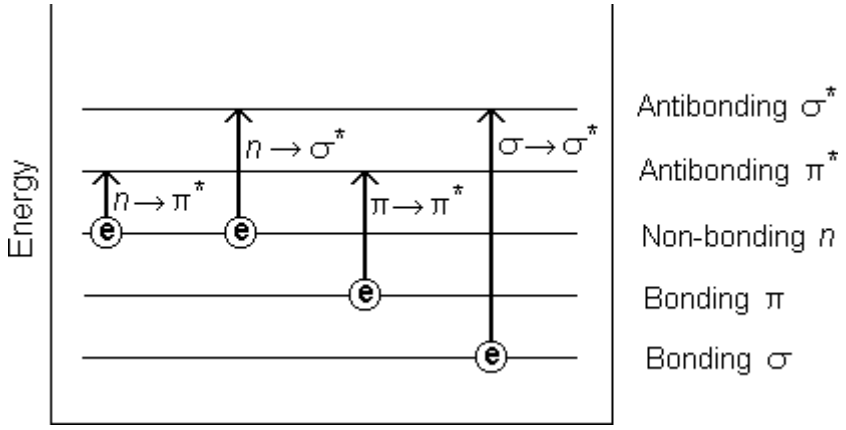


Fig no.1 Electronic Transition Process in a Molecule

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.

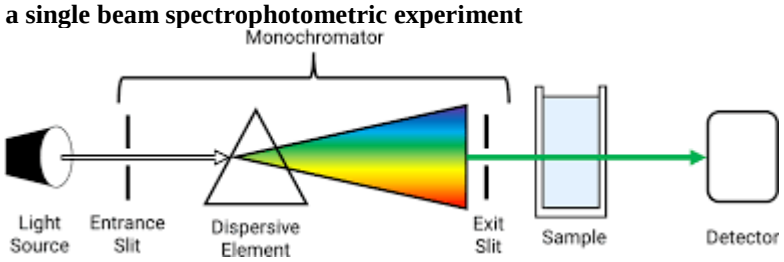
Absorbing species containing л, σ and n electrons

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**
- **n-σ* Transitions**
- **n-σ^* and л-π^* Transitions**

Fig. 2: Instrumentation.

Schematic diagram of a single beam spectrophotometric experiment



Schematic diagram of a double beam spectrophotometer

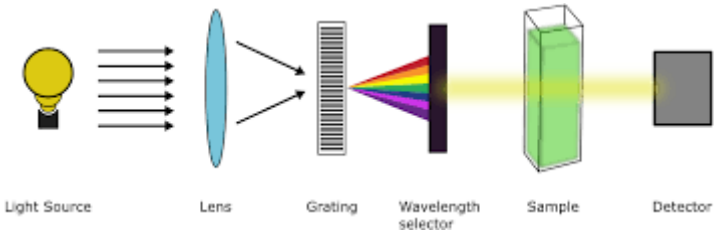


Fig. 3: Instrumentation of single and double beamspectrophotometer.

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 andvisible-NIR regions.

The Monochromator

Accepts Polychromatic Input Light from lamp and outputs monochromatorLight.

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 DevicePrism

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 betransparent 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

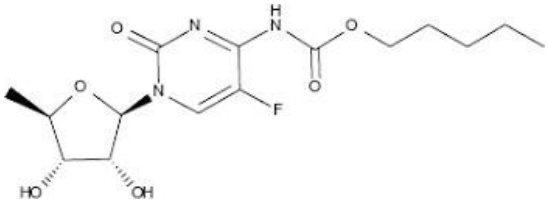
Photon Transducers: Light→ Electric current Photomultiplier tube

Photoconductivity transducersPhotovoltaic cells

Silicon photodiode

Drug Profile of Capecitabine

Structure



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

3. EXPERIMENTAL WORK
3.1 MATERIALS AND METHODS
3.1.1. Equipment/Instrument details.
Table 1: List of Equipment / Instrument details.

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

3.1.2Chemicals and Reagents
Table 2: list of Chemicals and Reagents.

S. No.	Name	Manufacturer	Grade
1	Capecitabine Working standard	Cipla Pharmaceuticals	--
2	Capecitabinetablets	Ranbaxy pvt. LTD	--
3	Battery Water	Milli-pore	Milli-Q
4	0.45mm PVD filter	Rankem	D004A07

3.2. Analytical Method Development
A. Selection of wavelength
A solution of 100µg/mL of Capecitabine was prepared in battery water. The resulting solutions were scanned individually in UV-Visible spectrophotometer from 190 to 400 nm. The optimal response for the drug was obtained at 240 nm. Hence the complete method was processed at the wavelength of 240 nm. The solvent selected to dissolve the drug was battery water because of its favourable UV transmittance, low viscosity and freesolubility.

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 battery water 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 beforetaking 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: Battery water 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 themark with battery water.

Preparation of Level - II (10ppm of Capecitabine)

1.0ml of stock solution was taken in 10ml of volumetric flask diluted up to themark with diluent.

Preparation of Level-III (15ppm of Capecitabine)

1.5ml of stock solution was taken in 10ml of volumetric flask diluted up to themark with diluent.

Preparation of Level - IV (20ppm of Capecitabine)

2.0ml of stock solution was taken in 10ml of volumetric flask diluted up to themark with diluent**.**

Preparation of Level - V (25ppm of Capecitabine)

2.5ml of stock solution was taken in 10ml of volumetric flask diluted up to themark with diluent.

Procedure

Each level solution was scanned in UV spectrophotometer at 240nm and absorbance was measured. A graph of peak area versus concentration (on X- axis 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 andis given in the Fig.No.5

The results are discussed in

Table 3: Acceptance criteria

1. Correlation Coefficient should be not less than 0.9990.
2. % RSD of peak area's 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 240mm 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 Assayconcentration)

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 Assayconcentration)

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 upto 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 ofthe 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 a10ml 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 wasfound 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% byboth 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 preparationsshould be not more than 2.0 %.

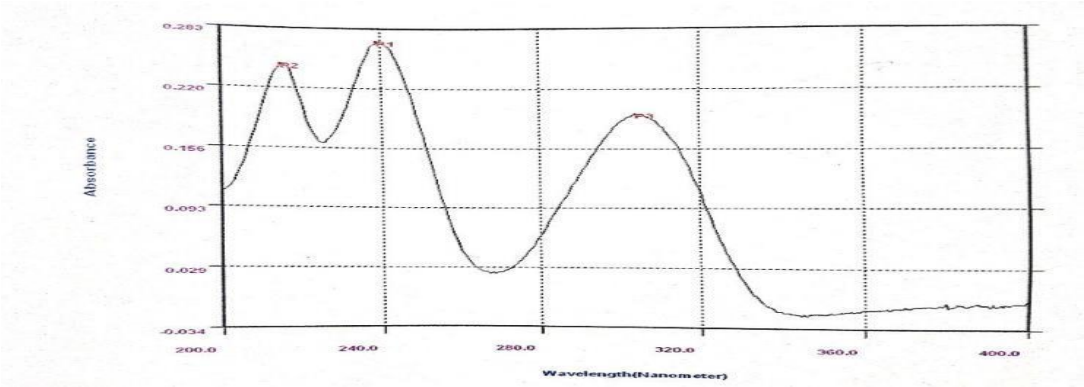
6. Robustness
The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like composition, temperature variations which may differ but the responses were still within the specified limits of the assay.

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 240 nm. Hence, the complete method was processed at

the wavelength of 240nm. The spectrum is shown in Fig.No.4.



UV spectrum of capecitabine in distilled water

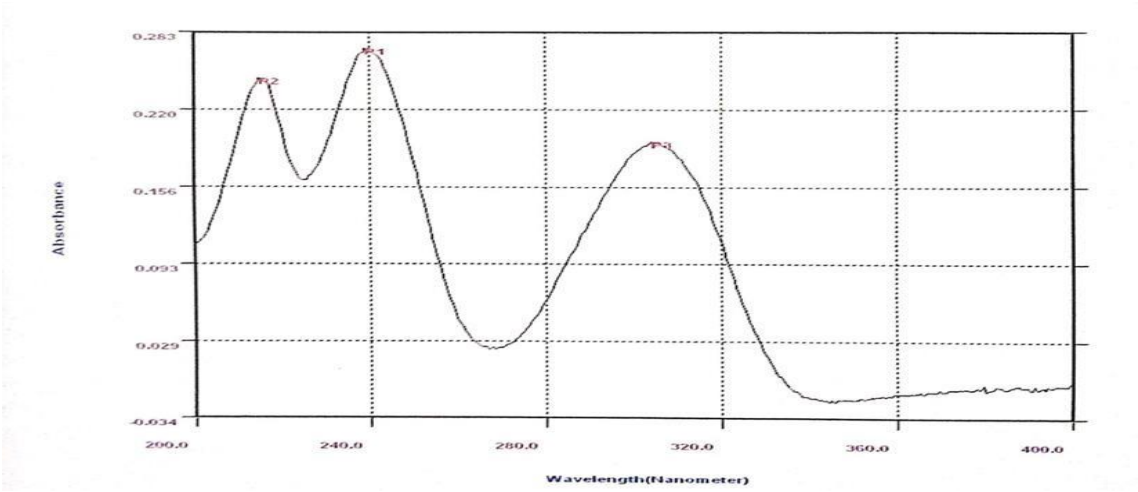
4.1.3. Method Validation

1. Specificity

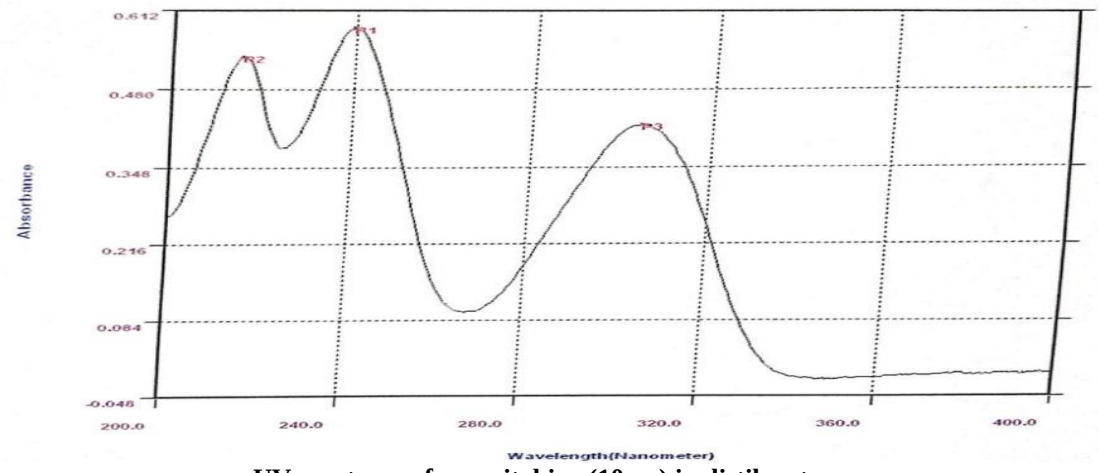
THE absorbance value of standard and sample are identical with nearly samevalues. Hence, the given drug passes specificity.

2. Linearity

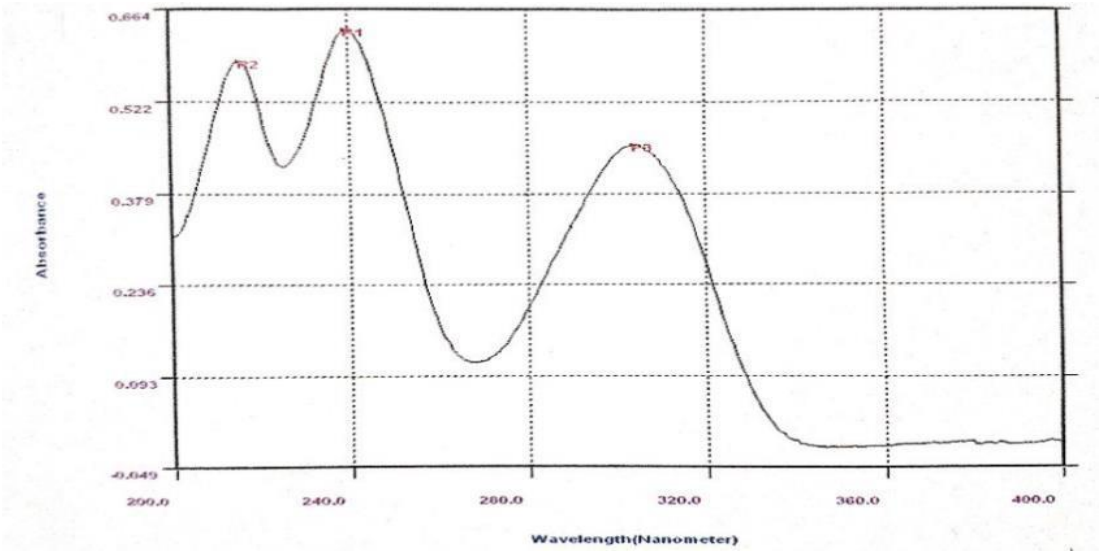
From the Linearity data it was observed that the method was showing linearity in the concentration range of 5-25 ug/ml. Correlation coefficient was found to be 0.999. The data of linearity is illustrated in Table no.3. The spectrums for the linearity are presented and the linearity curve is plotted and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



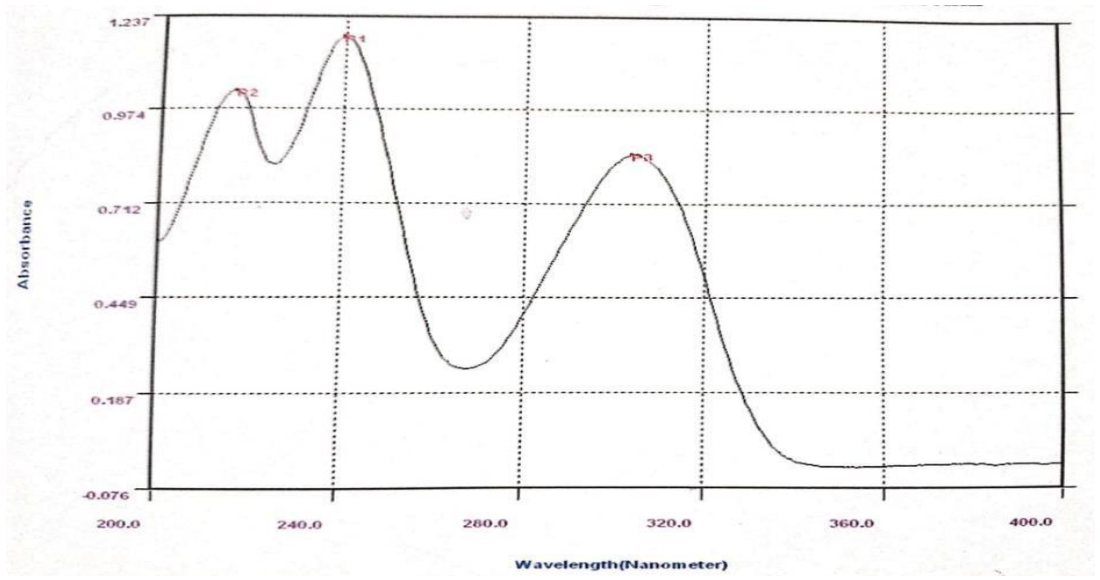
UV spectrum of capecitabine (5mg) in distilled water at 240nm



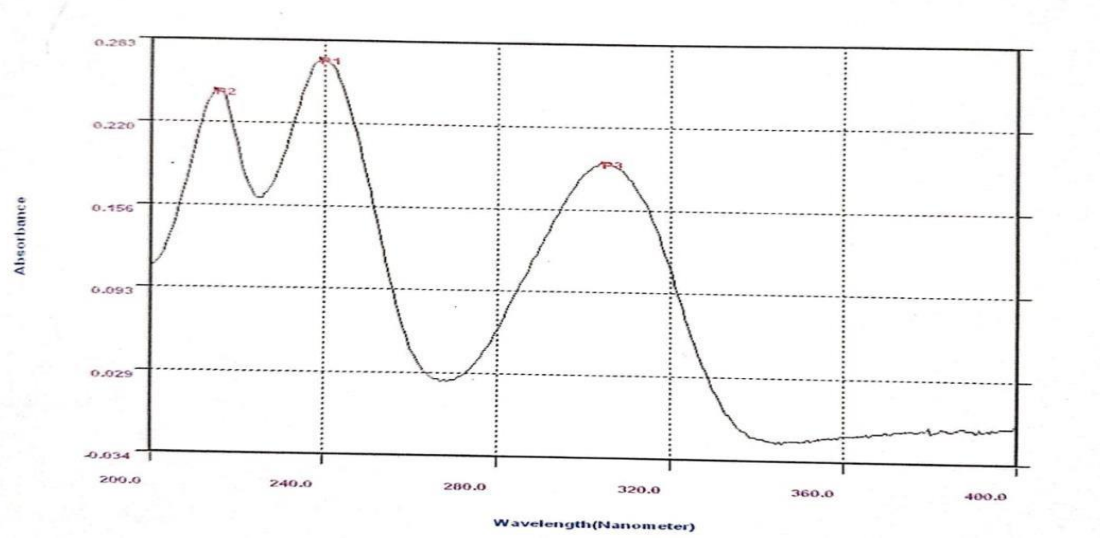
UV spectrum of capecitabine (10mg) in distil water



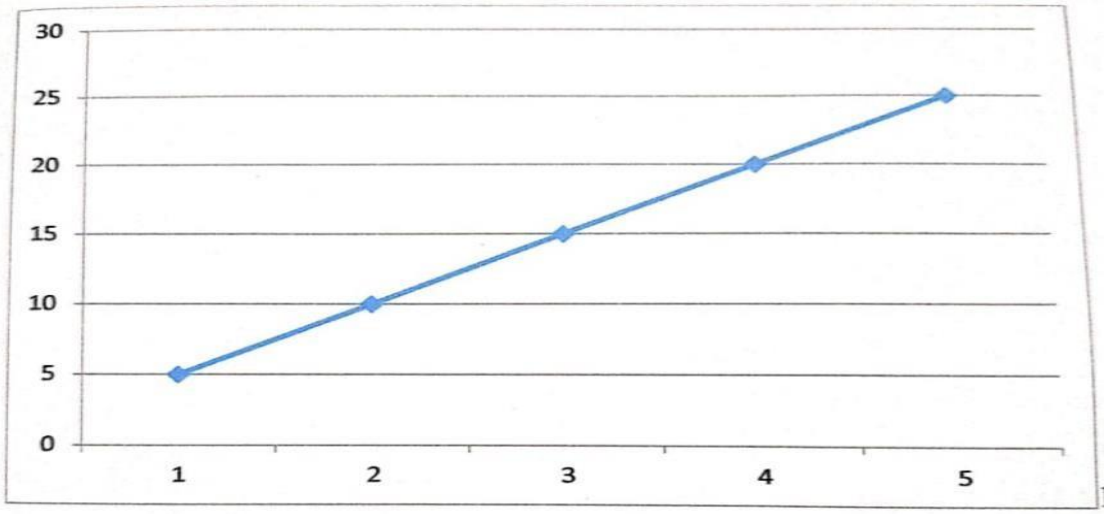
UV spectrum of capecitabine (15mg) in distil water



UV spectrum of capecitabine (20mg) in distil water



UV spectrum of capecitabine (25mg) in distil water



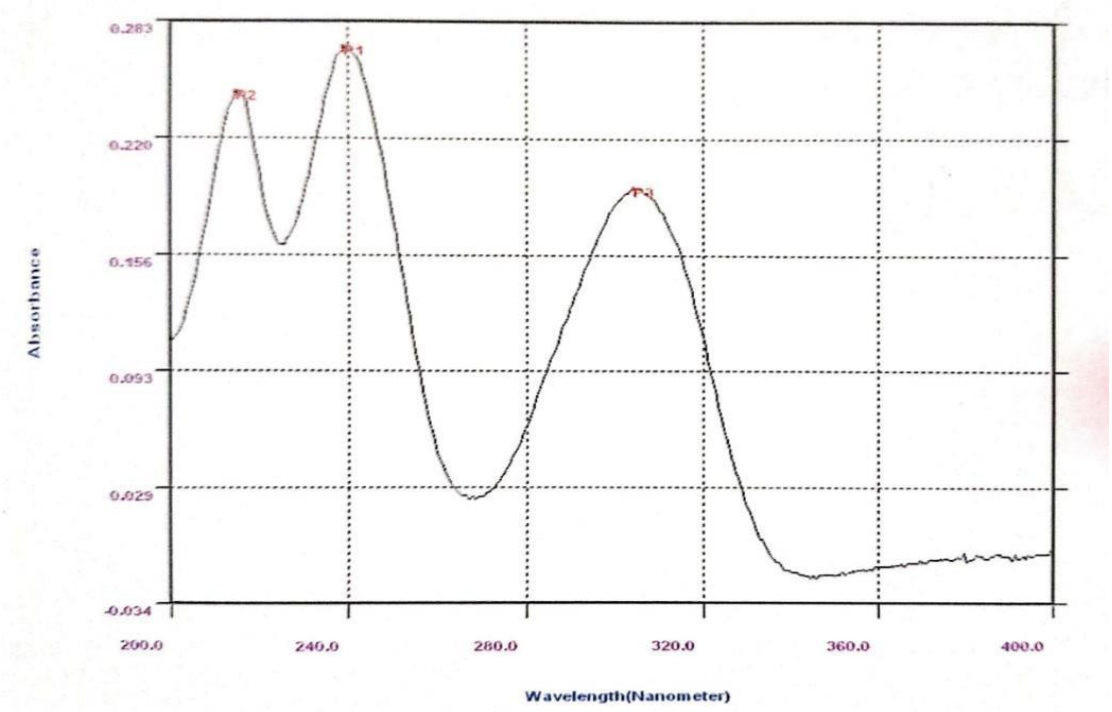
Calibration curve of Capecitabine
Correlation Coefficient 0.997
Table 3: Linearity results for Capecitabine.

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

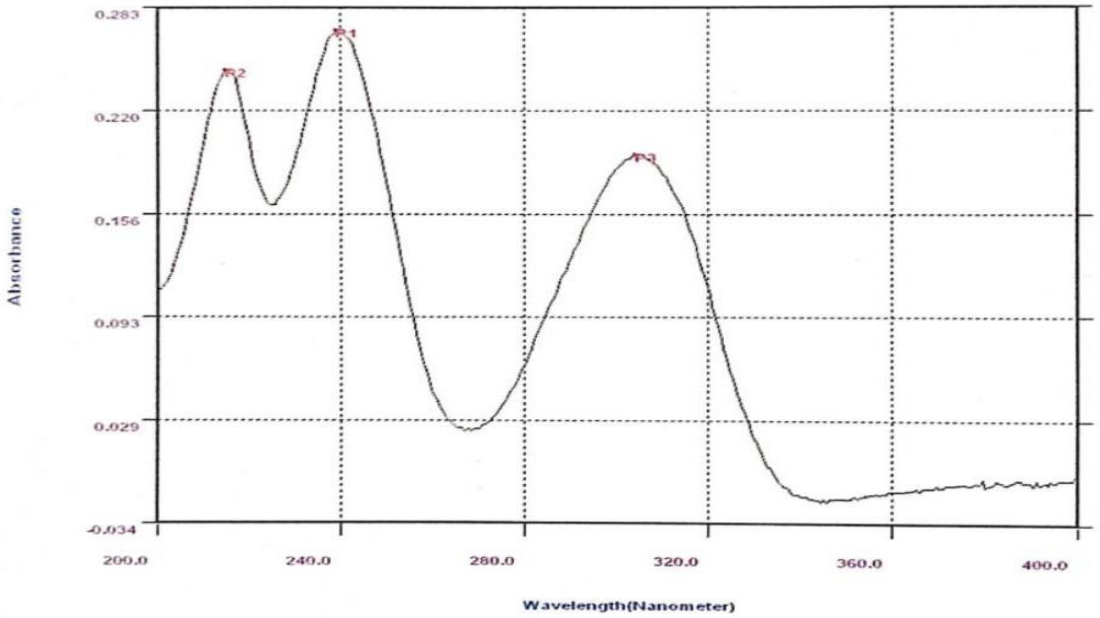
Results: correlation coefficient of capecitabine was found to be **0.995[NMT0.999]**

3. Accuracy

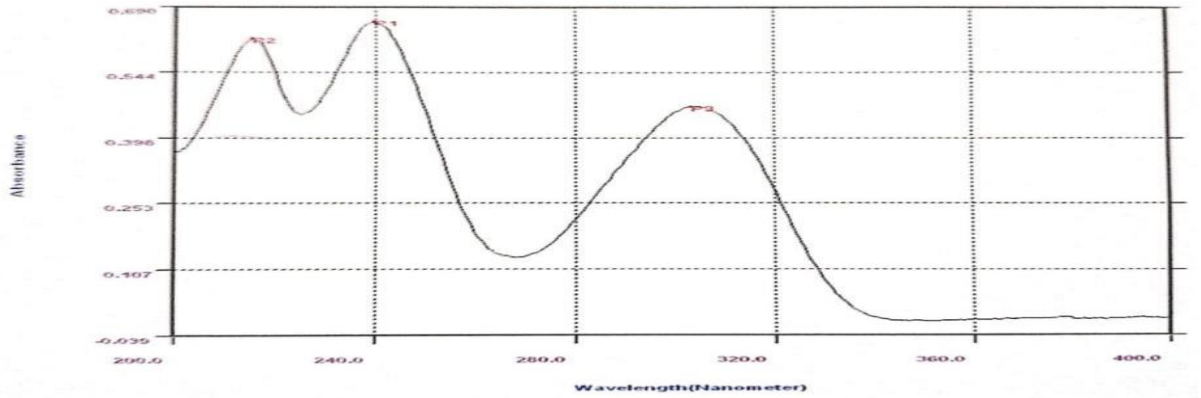
The recoveries of pure drug from the analysed solution of formulation were 99.22 % to 100.11%, which shows that the method was accurate. The summary of Accuracy results were expressed in Table no.4. And spectrums for accuracy were shown in Fig.No.6.-8



Spectrum for Accuracy50% Conc



Spectrum for Accuracy100% Conc



Spectrum for Accuracy150% Conc

Table 4: %Recovery Results for CapecitabineResults.

SampleNo.	SpikeLevel	Amount(mg/ml)added	Amount(mg/ml)found	%Recovery	Mean% Recov ery
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%	

The % Recovery for 100% Accuracy level of Capecitabine was found to be 99.4% (98.0 to 102.0%) Fig.No. 9.

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

A) Repeatability

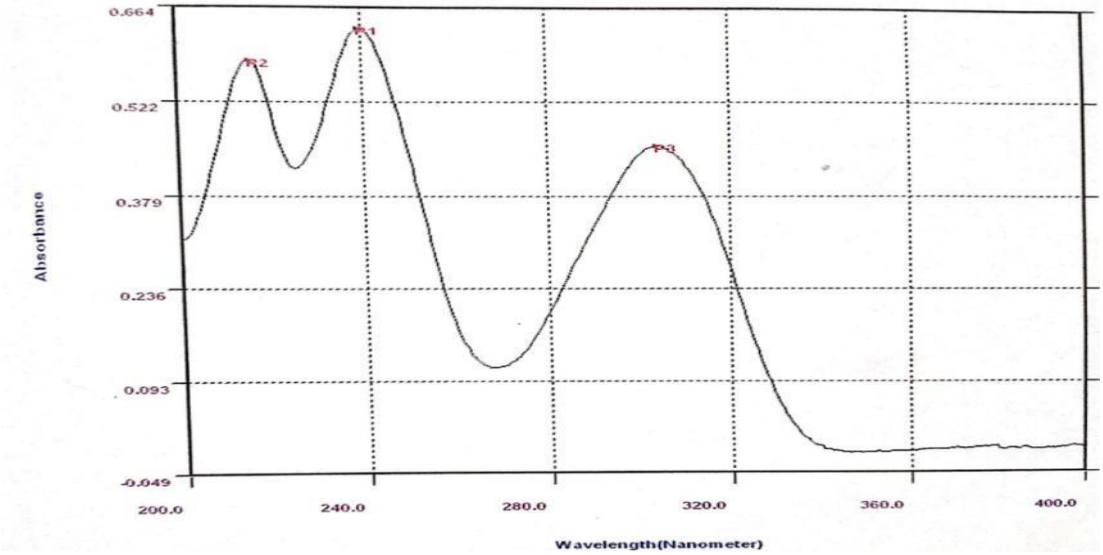


Fig. 9. a; Sample spectrum for Repeatability (15ppm)

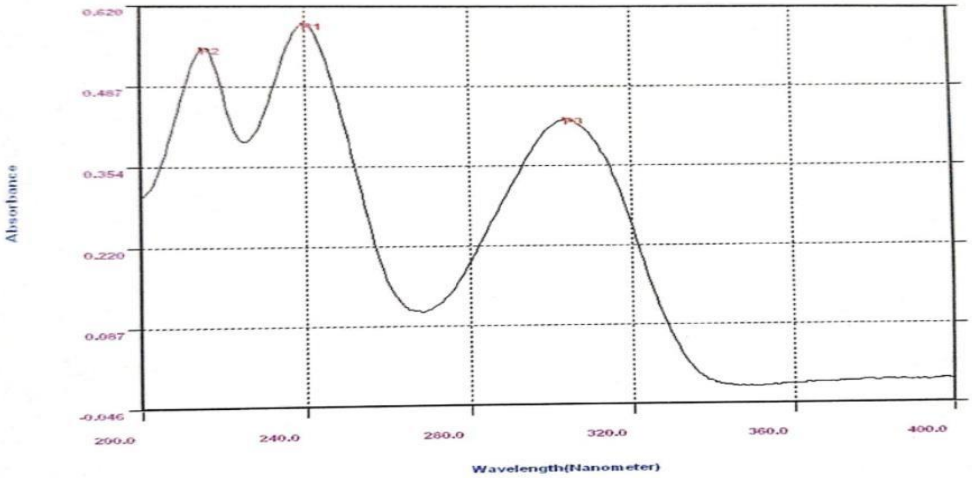


Fig. 9.b; Sample spectrum for Repeatability (15ppm)

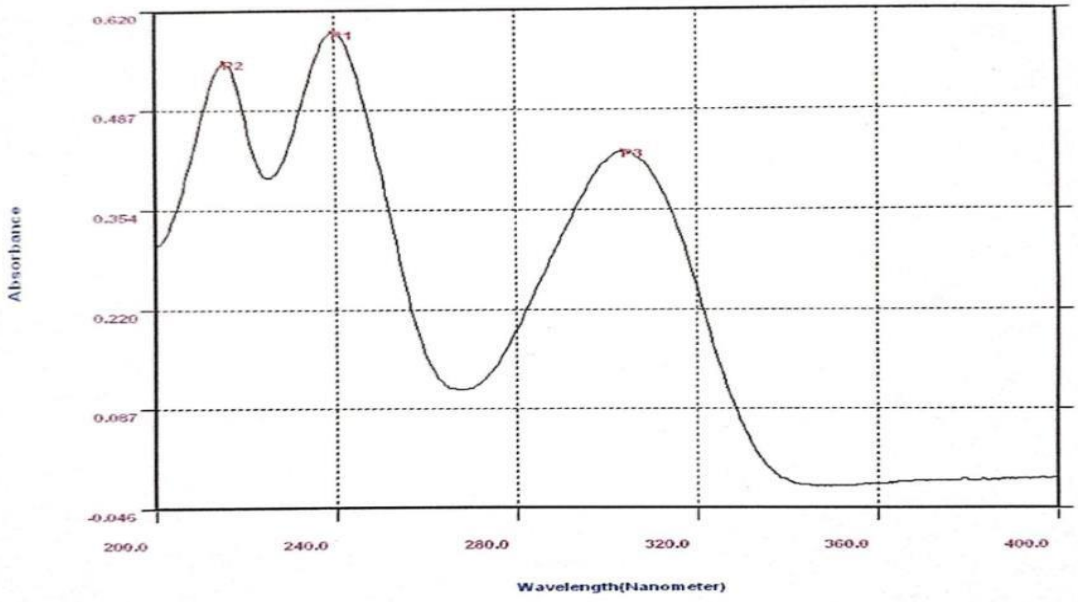


Fig. 9.c; Sample spectrum for Repeatability (15ppm)

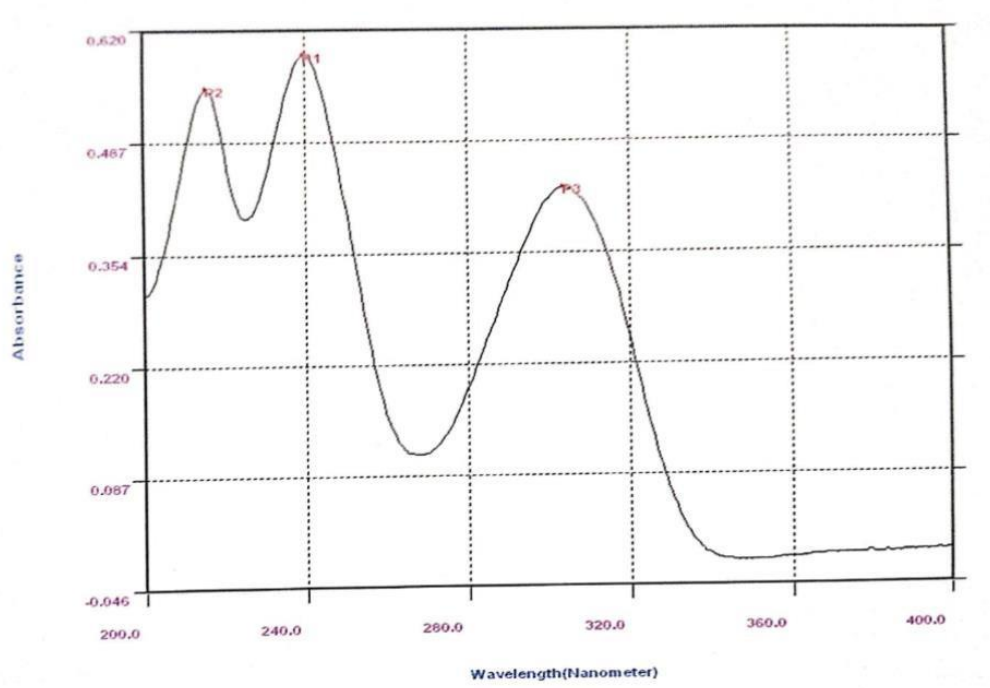


Fig. 9.d: Sample spectrum for Repeatability (15ppm)

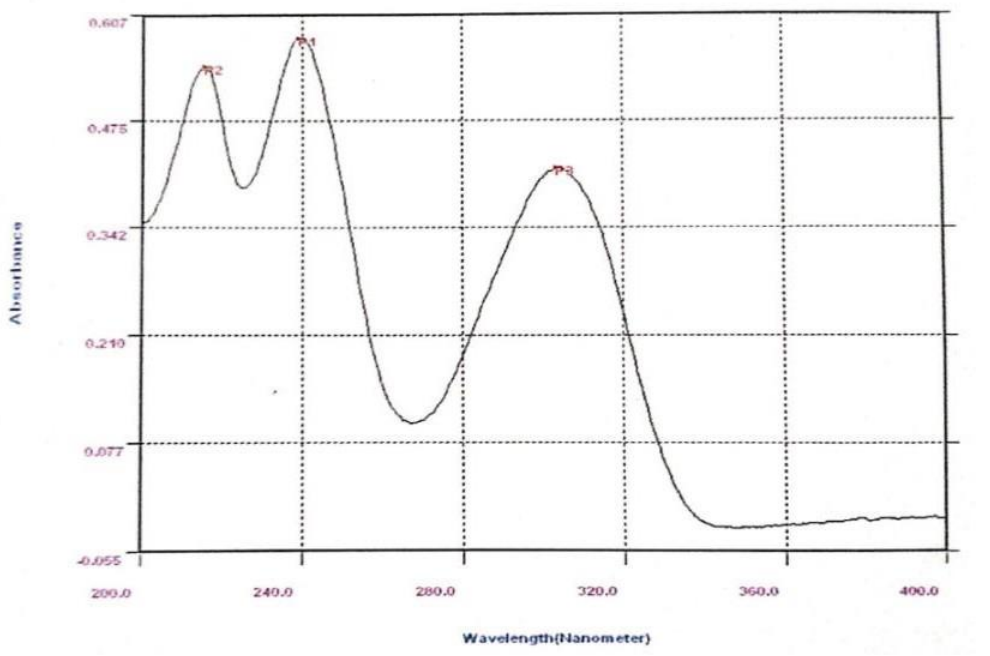


Fig. 9.e: Sample spectrum for Repeatability (15ppm).

Table 5: Sample spectrum value for Repeatability of Capecitabine.

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

Result: The % RSD for the area of five spectra results of Capecitabine wasfound to be **0.42. (NMT 2).**
Intermediate precision (Analyst to Analyst variability) or Rugeddness; Analyst2.

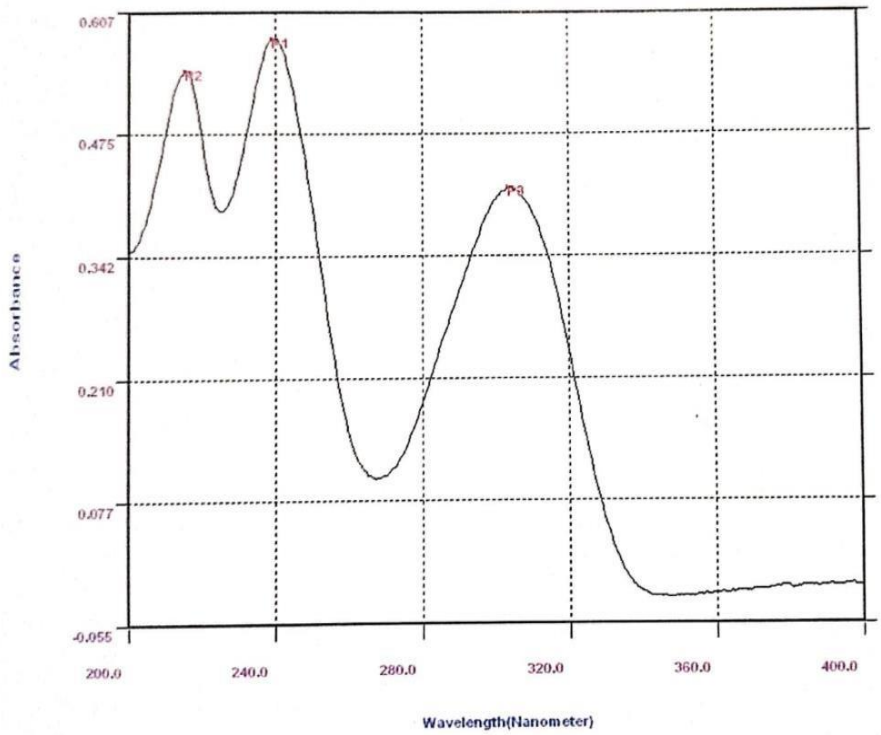


Fig. 10.a: Spectrum for intermediate precision.

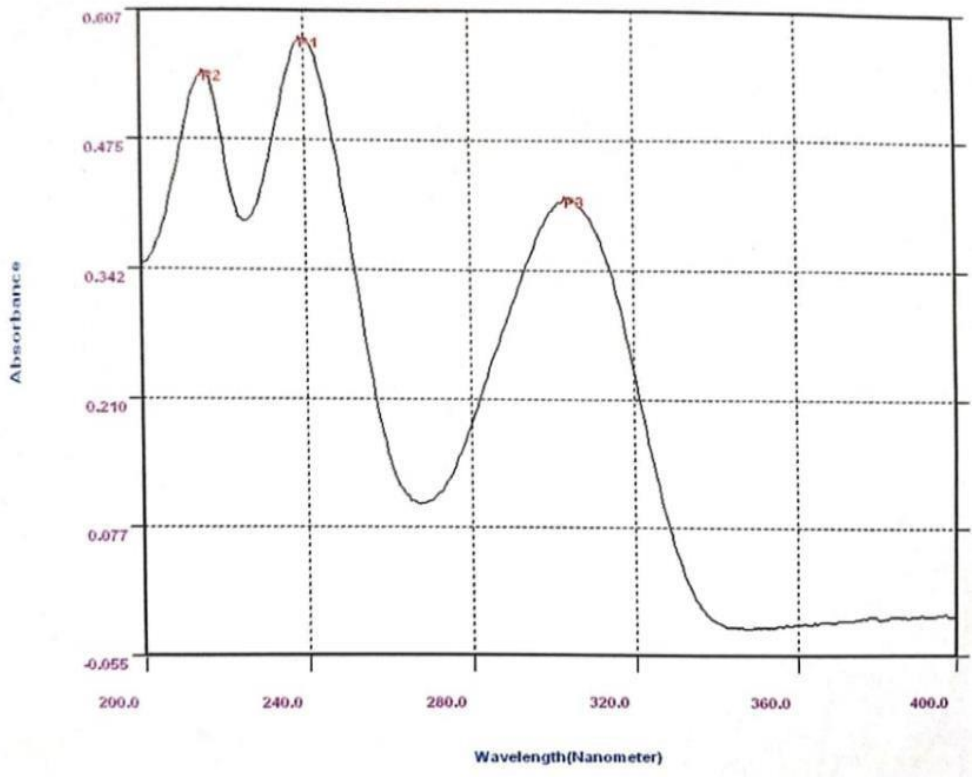


Fig. 10.b: Spectrum for intermediate precision.

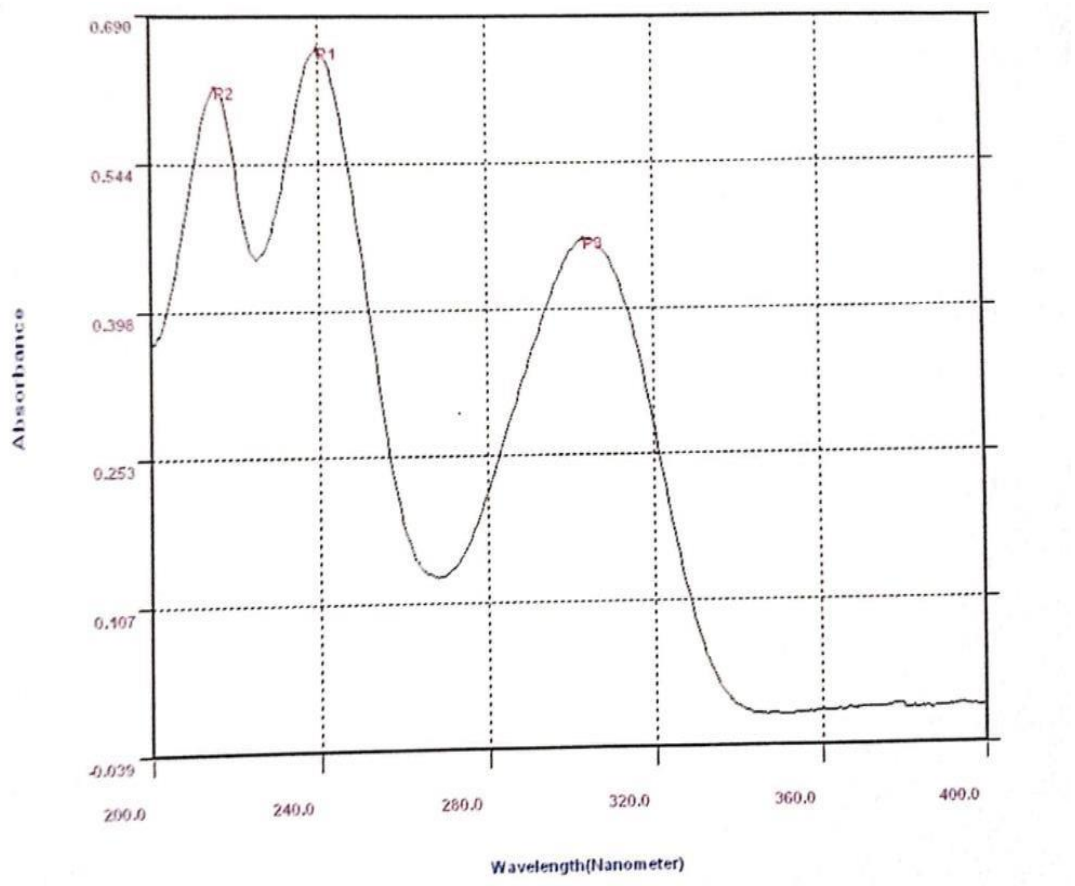


Fig. 10.c: Spectrum for intermediate precision.

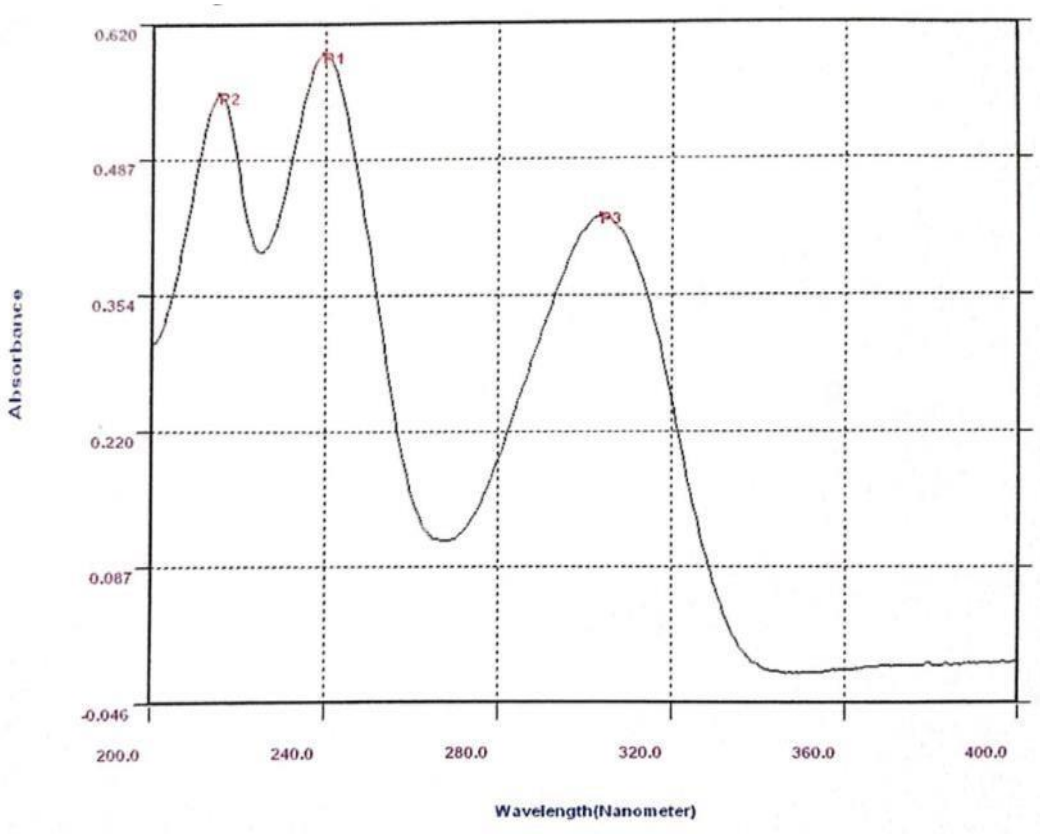


Fig. 10.d: Spectrum for intermediate precision.

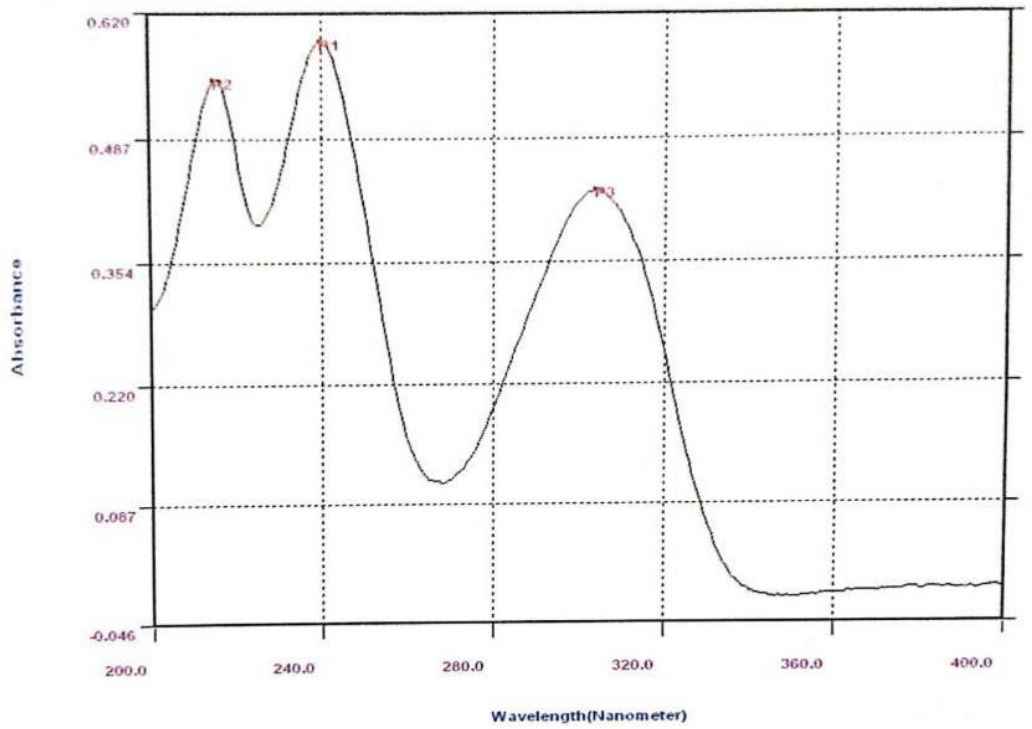


Fig. 10.e: Spectrum for intermediate precision.

Table 6: Sample spectrum value for intermediate precision ofcapecitabine.

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

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

4.2. CONCLUSION

For routine analytical purpose it is desirable to establish methods capableof 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 powerfuland convenient analytical tool.

Capecitabine was freely soluble in ethanol, methanol, acetonitrile and in distilled water. Distilled water 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 proposedmethod 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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