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UV spectrophotometric stability indicating method development and validation for the estimation of 5-fluorouracil in the bulk

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Abstract

For the determination of the 5-fluorouracil in the bulk, a simple, accurate, precise, stability-indicating UV spectrophotometric approach has been established. For determination of 5-fluorouracil HPLC grade water used as a solvent and shows maximum absorbance at 265 nm. The developed approach completed validation for a variety of parameters, such as linearity, precision, accuracy, robustness, ruggedness, LOD, and LOQ. This method followed Beer's law for the concentration range of 4-14 µg/ml for 5-fluorouracil. The recovery testing showed that the suggested approach was accurate and per cent relative standard deviation (%RSD) confirmed this method was precise, robust. Forced degradation studies were performed under acidic, alkali, thermal and oxidative conditions as per ICH guidelines.

1. Introduction

In fact, 5-fluorouracil is the third most often used chemotherapy drug for treating solid tumors worldwide (Sara *et al.,* 2018*)*.The chemical name of 5- fluorouracil is 5- fluro-1H,3H-pyrimidine-2,4-dione. 5-fluorouracil is used as a anticancer agent (Figure 1). Inhibition of thymidylate synthase and integration of its byproducts into RNA and DNA are the primary mechanisms of action of 5-fluorouracil (Longley *et al.,* 2003). The 5-fluorouracil is being used as an antimetabolic in the topical treatment of various skin diseases with emphasis on skin cancer, vitiligo and psoriasis. Tegafur [5-fluoro-1-(2-tetrahydrofuryl)-2,4(1H,3H)-pyrimidinedione] is a prodrug of 5-fluorouracil (5-FU) and it is converted into 5-FU by cytochrome P450 enzymes (Yamamiya *et al.,* 2013). The principal contaminants 5-FU and N-1(2-furanidyl) uracil must be identified for the quality control of tegafur raw materials (Badea *et al.,* 2002).

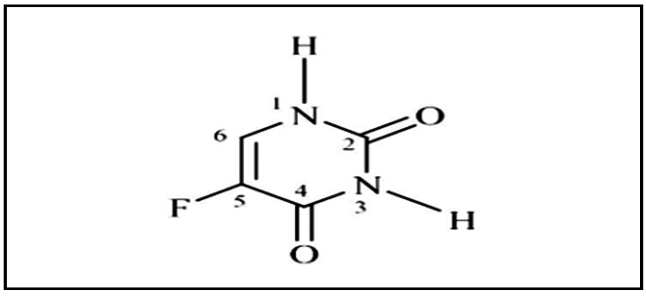

Figure 1: Chemical structure of 5-flourouracil.

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Literature review revealed that till now there was only one UV spectrophotometric method was reported for determination of 5-fluorouracil (Ileana *et al.,* 2012). In comparison to the reported method, the proposed UV spectroscopic method was shown to be more cost-effective and sensitive.

2. Materials and Methods

2.1 Instruments

A UV/Visible spectrophotometer (Labindia) and 1cm UV matched quartz cells were used for the validation of 5-fluororacil in the bulk.

2.2 Materials (Shwetha *et al.,* 2020)

5-fluorouracil standard drug was purchased from Yarrow Chemicals Private Limited, India. The analytical grade chemicals and reagents that were used were all procured. Throughout the work, calibrated glassware was utilised.

2.3 Selection of suitable solvent (Sai Krupa Raj *et al.,* 2022)

About 20 mg of 5-fluorouracil were weighed, and the solubility of the drug in water, 0.1 N NaOH, dimethylformamide (DMF), and HPLC grade water was examined. The drug was found to be soluble in HPLC grade water by sonication for 5 min. It was selected throughout study because of obtaining sharp peak at the selected wavelength.

2.4 Standard stock solution preparation

20 mg of 5-fluorouracil weighed accurately, was then transferred to a 50 ml volumetric flask and dissolved in HPLC grade water using a sonicator for up to five minutes. Then final volume was adjusted with the same HPLC grade water to get final concentration of 400 µg/ml. From the above concentration, pipette out 5 ml of solution

and diluted with the help of HPLC grade water in 50 ml volumetric flask to get 40 µg/ml.

2.5 Working standard solution preparation

The standard stock solution was diluted appropriately to get the final concentration of 8 µg/ml.

2.6 Selection of wavelength for analysis

The wavelength for analysis of 5-flurouracil was selected from the UV- spectrum. To find out the λmax, 20 µg/ml concentration of 5-fluorouracil was prepared and it was scanned in the UV range between 200-400 nm. The absorbance maxima (λmax) was found at 265 nm against HPLC grade water (Figure 2).

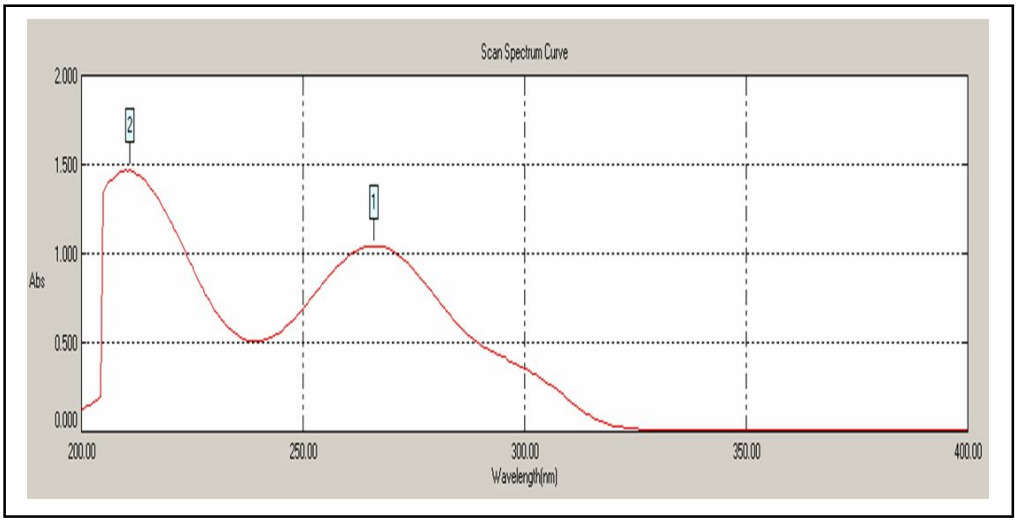

Figure 2: UV spectra of 5-fluorouracil.

3. Results

3.1 Validation parameters

The proposed method was validated in accordance with ICH guidelines (ICH, 2005).

3.1.1 Linearity

By examining various concentrations of standard solutions of 4-14 µg/ml and plotting absorbance against concentrations of the analyte (Figure 3), the suggested UV spectroscopic method linearity was assessed. Six distinct concentrations in the range between 4-14 µg/ml of 5-fluorouracil were prepared from the standard stock solution and examined at a wavelength of 265 nm and results were displayed in Table 1. The regression coefficient (R^2) value was found to be 0.999.

Table 1: Linearity results

Concentration (µg/ml)	Absorbance
4	0.240
6	0.362
8	0.469
10	0.586
12	0.698
14	0.805

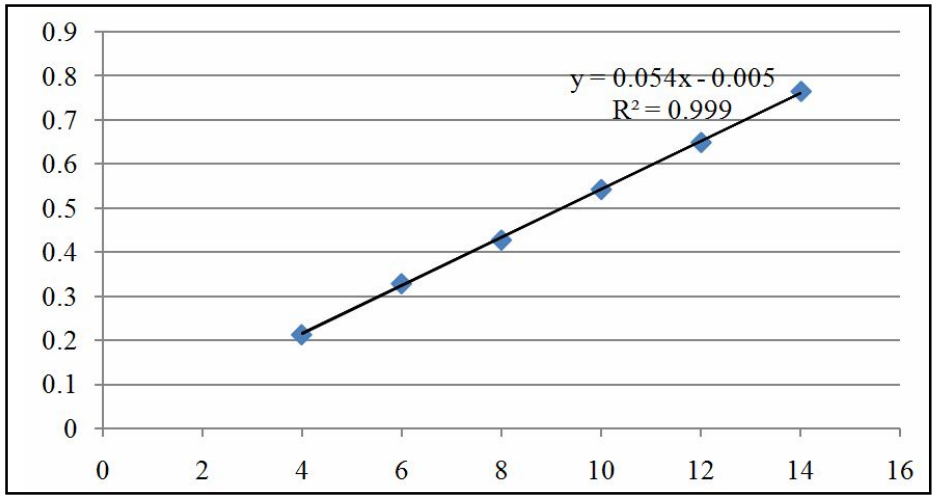

Figure 3: Linearity curve for 5-fluorouracil.

3.1.2 Accuracy

Recovery trials at three distinct API levels (50, 100, and 150%) were performed using the standard addition method. Accuracy is determined by calculating % recovery and mean % recovery. It was found to be in the range between 98-102% (Table 2).

Table 2: Results for accuracy

% level of accuracy	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	Mean % recovery
50%	4	3.964	99.1	98.51
50%	4	3.947	98.67	
50%	4	3.911	98.51	
100%	8	8.105	101.31	98.30
100%	8	7.858	98.6	
100%	8	7.894	98.22	
150%	12	11.057	92.141	98.06
150%	12	12.337	102.8	
150%	12	11.911	99.25	

3.1.3 Precision

Precision was carried out by performing interday and intraday variation. Repeatability measurements was carried out by analyzing six different solutions containing same concentration of 5-fluorouracil and % RSD was calculated and it was found to be <2 (Tables 3 and 4).

Table 3: Results for inter-day precision

	Inter-day precision		
Concentration	**Day- 1**	**Day -2**	**Day -3**
8 µg/ml	0.494	0.512	0.498
8 µg/ml	0.499	0.515	0.484
8 µg/ml	0.500	0.515	0.482
8 µg/ml	0.504	0.512	0.488
8 µg/ml	0.506	0.504	0.491
8 µg/ml	0.494	0.508	0.849
Average	**0.499**	**0.512**	**0.487**
SD	**0.0045**	**0.0065**	**0.0048**
% RSD	**0.901**	**1.268**	**0.9841**

Table 4: Results for intra-day precision

	Intra-day precision	
Concentration	**Morning**	**Evening**
8 µg/ml	0.491	0.546
8 µg/ml	0.498	0.545
8 µg/ml	0.499	0.523
8 µg/ml	0.500	0.530
8 µg/ml	0.504	0.536
8 µg/ml	0.512	0.540
Average	**0.501**	**0.541**
SD	**0.006368**	**0.00610**
% RSD	**1.27**	**1.12**

3.1.4 Ruggedness

Six replicates of the working standard solution were used to examine analyst to analyst variation for ruggedness studies. The % RSD values were found to be < 2 (Table 5).

Table 5: Results for ruggedness

Analyst-1		Analyst-2	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
8 µg/ml	0.460	8 µg/ml	0.455
8 µg/ml	0.462	8 µg/ml	0.456
8 µg/ml	0.465	8 µg/ml	0.449
8 µg/ml	0.469	8 µg/ml	0.452
8 µg/ml	0.464	8 µg/ml	0.459
8 µg/ml	0.468	8 µg/ml	0.456
Average	**0.465**	**Average**	**0.455**
SD	**0.003147**	**SD**	**0.003207**
% RSD	**0.677**	**% RSD**	**0.705**

3.1.5 Robustness

To establish that the robustness of the method, six replicates of working standard solution were prepared and absorbance was checked at variable method condition like using different wavelengths (max ± 2nm). The % RSD values were found to be < 2 (Table 6) and it indicated that the developed method was robust.

Table 6: Results for robustness

Concentration	263 nm	265 nm	267 nm
8 µg/ml	0.455	0.462	0.461
8 µg/ml	0.455	0.468	0.467
8 µg/ml	0.450	0.464	0.463
8 µg/ml	0.455	0.465	0.465
8 µg/ml	0.459	0.461	0.461
8 µg/ml	0.459	0.465	0.464
Average	**0.457**	**0.464**	**0.464**
SD	**0.001863**	**0.002267**	**0.002141**
% RSD	**0.407%**	**0.488%**	**0.462%**

3.1.6 Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) values were used for describe the method sensitivity. It was calculated by taking the slope and standard deviation of response from calibration curve of analyte, which were for determining the linearity. It is done by using following formulas and results were found to be < 1 (Table 7), so the proposed method was sensitive.

LOD = 3.3 × Standard deviation of intercept/Slope

LOQ = 10 × Standard deviation of intercept/Slope

Table 7: Results for sensitivity

API	Limit of detection	Limit of quantification
5-Fluorouracil	0.206 µg/ml	0.625 µg/ml

3.1.7 Solution stability

It was done by using a working standard solution. After keeping 24 h at room temperature, the prepared working standard solution was scanned in the UV range between 200-400 nm. After observing both UV spectrums, the solution found to be stable (Figures 4 and 5).

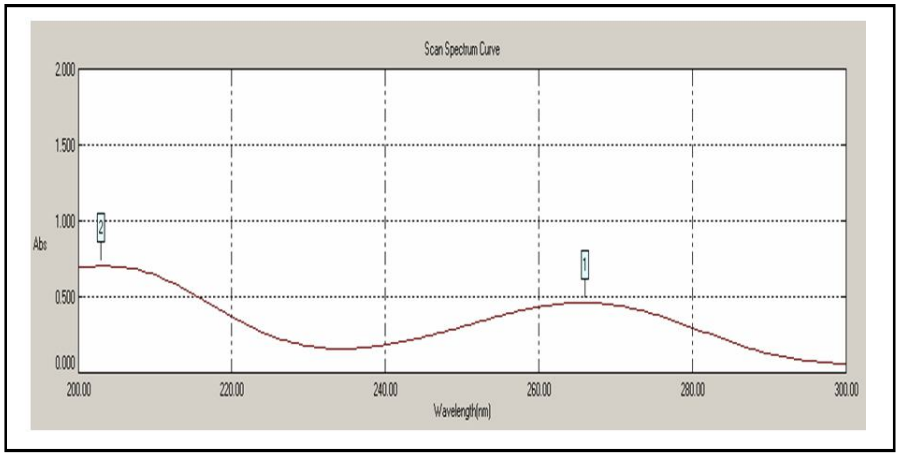

Figure 4: UV spectra of working standard solution at 0 h.

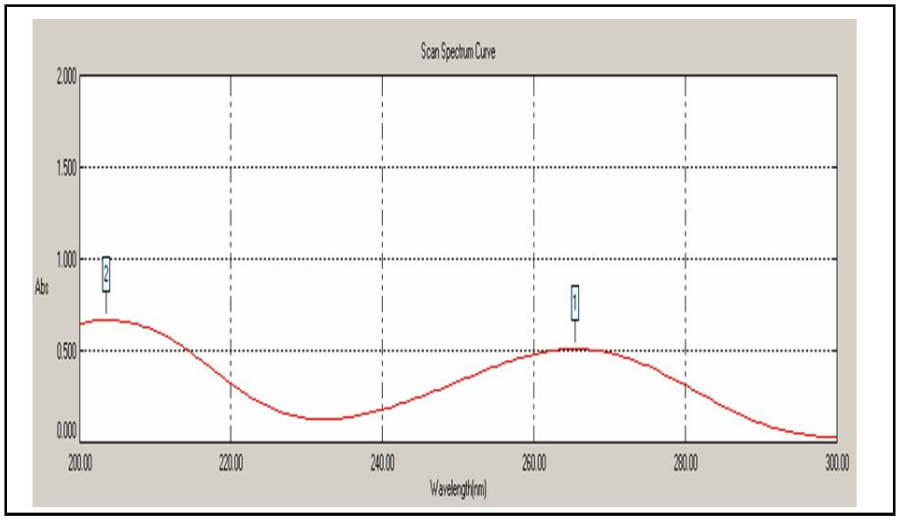

Figure 5: UV spectra of working standard solution after 24 h.

3.2 Forced degradation studies

The forced degradation studies were performed as per ICH guideline (ICH, 2003).

3.2.1 Acid induced degradation

An aliquot of 2 ml of 5-fluorouracil standard stock solution was carried into 10 ml volumetric flask and the solution was diluted with 0.1 N HCl to get final concentration of 8 µg/ml. It is subjected to heating (60^0C) under the hot air oven for 30 min till sufficient degradation. The degraded absorbance of exposed solution was recorded by scanning between 200-400 nm against blank (Figure 6). The % of drug recovered was calculated by using the following formula:

$$\% \text{ of drug recovered} = \frac{\text{Degraded absorbance}}{\text{Standard absorbance}} \times 100$$

$$= 0.415 \div 0.524 \times 100$$

$$= 0.79 \times 100$$

$$= 79\%$$

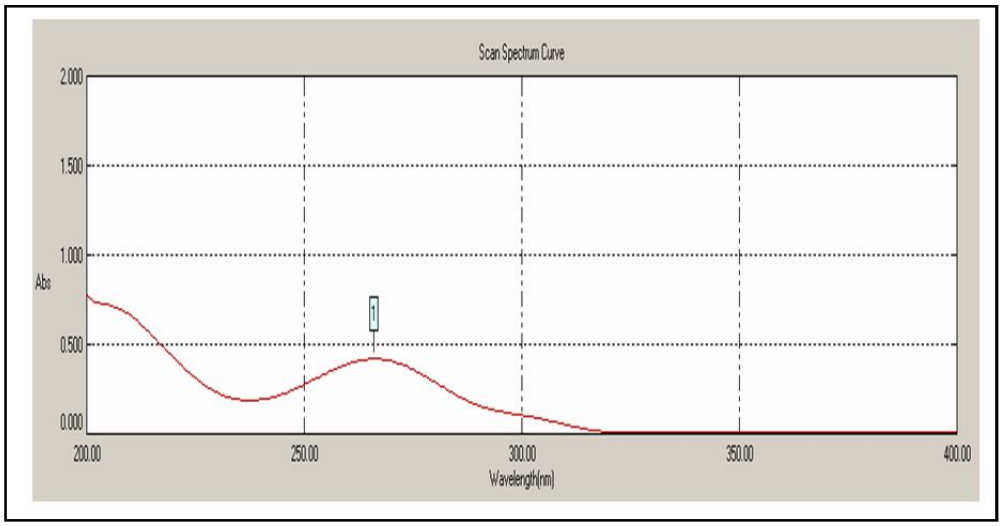

Figure 6: UV Spectra of acid degradation.

3.2.2 Alkali induced degradation

An aliquot of 2 ml of 5-fluorouracil standard stock solution was carried into 10 ml of volumetric flask and the solution was diluted with 0.1 NaOH to get a final concentration of 8 µg/ml. It is subjected to heating (60^0C) under the hot air oven for 30 min till sufficient degradation. The degraded absorbance of the exposed solution was recorded by scanning between 200-400 nm against a blank (Figure 7). The % of drug recovered was calculated by using the following formula:

$$\% \text{ of drug recovered} = \frac{\text{Degraded absorbance}}{\text{Standard absorbance}} \times 100$$

$$= 0.413/0.469 \times 100$$

$$= 0.880 \times 100$$

$$= 88.0\%$$

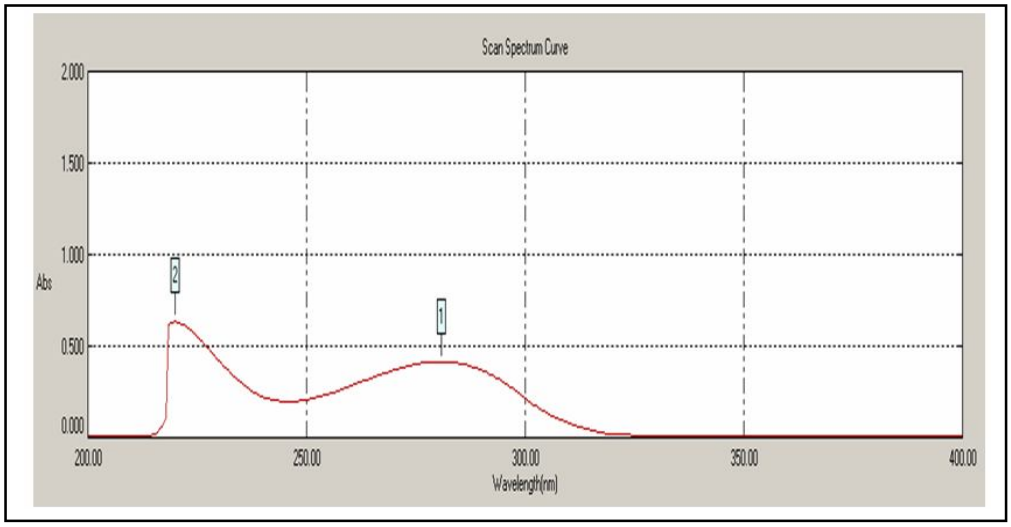

Figure 7: UV Spectra of alkali degradation.

3.2.3 Thermal degradation

An aliquot of 2 ml of 5-fluorouracil standard stock solution was carried into 10 ml of volumetric flask and then solution was diluted with HPLC grade water to get a final concentration of 8 µg/ml. It is subjected to heating (60^0C) under the hot air oven for 30 min till sufficient degradation. The degraded absorbance of the exposed solution was recorded by scanning between 200-400 nm against a blank (Figure 8). The % of drug recovered was calculated by using the following formula:

$$\% \text{ of drug recovered} = \frac{\text{Degraded absorbance}}{\text{Standard absorbance}} \times 100$$

$$= 0.408/0.469 \times 100$$

$$= 86.99\%$$

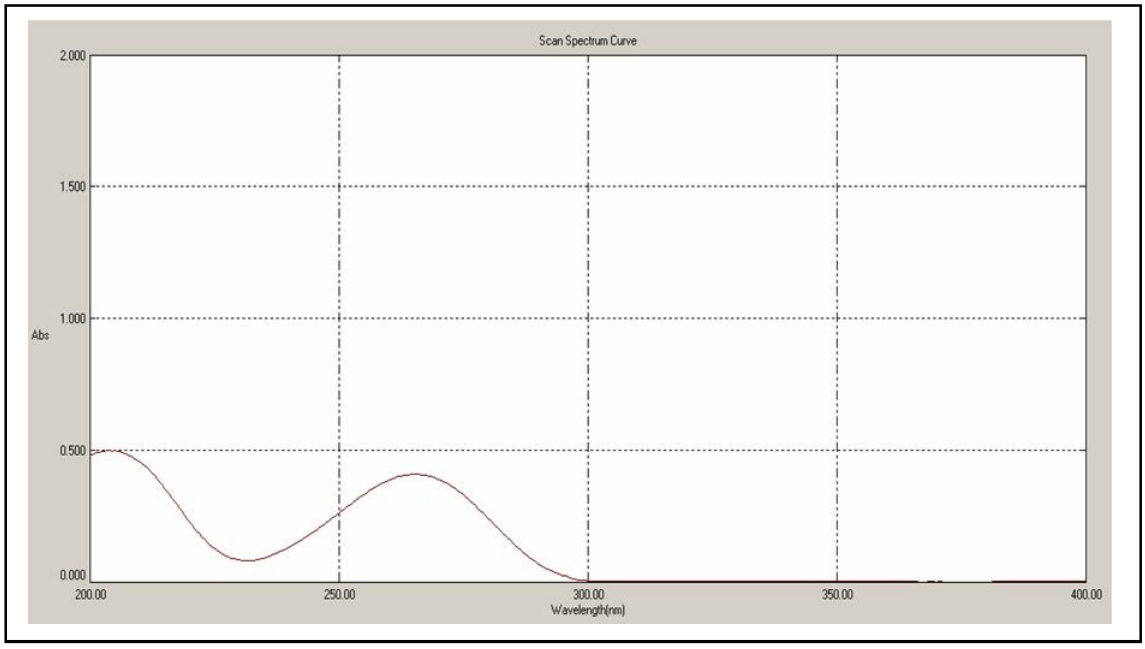

Figure 8: UV spectra of thermal degradation.

3.2.4 Photolytic degradation

Regarding photolytic degradation about 8 µg/ml solution was prepared and exposed to direct sunlight for 1 h. Absorbance was recorded at 265 nm on a UV spectrophotometer against HPLC grade water as a blank (Figure 9). The % of drug recovered was calculated by using the following formula:

$$\% \text{ of drug recovered} = \frac{\text{Degraded absorbance}}{\text{Standard absorbance}} \times 100$$

$$= 0.318/0.469 \times 100$$

$$= 67.80\%$$

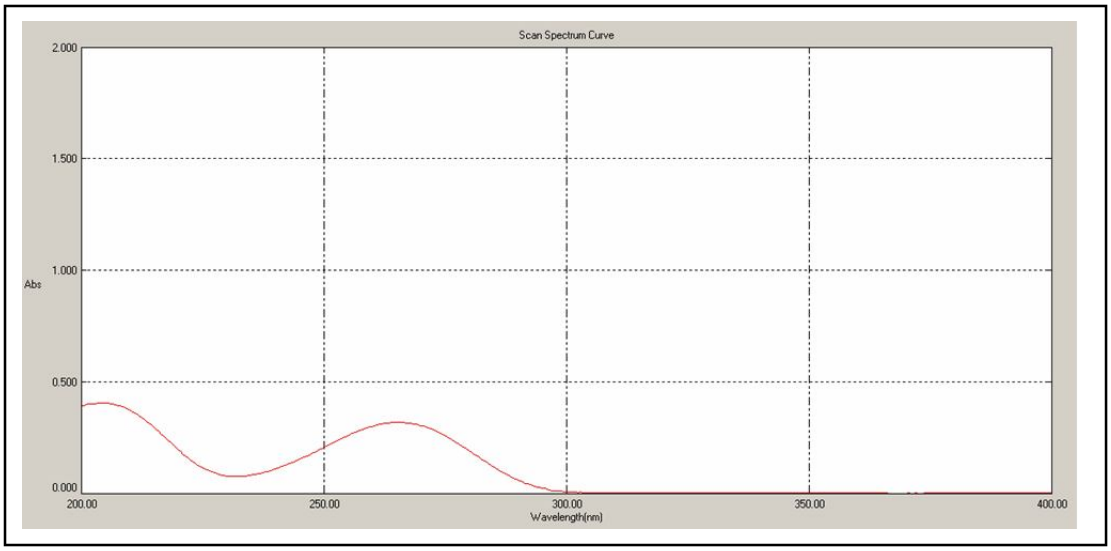

Figure 9: UV Spectra of photolytic degradation.

Table 8: Results of stability studies

Parameter	Exposure period	% Recovered	% Degraded
Acidic degradation	60^{0}C/30 min	79%	21%
Alkali degradation	60^{0}C/30 min	88.0%	12%
Thermal degradation	60^{0}C/30 min	86.99%	13.01%
Photolytic degradation	1 h for sunlight	67.80%	32.20%

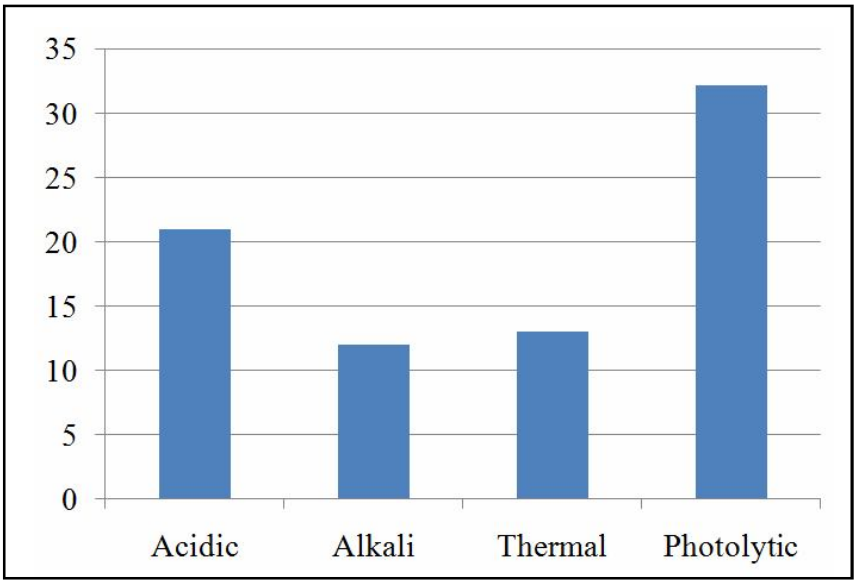

Figure 10: Per cent degraded absorbance in forced degradation studies.

4. Discussion

To optimize the method by UV spectrophotometer for 5-fluorouracil was tested with different solvents such as 0.1 N NaOH, dimethylformamide, and HPLC grade water. Due to great solubility and reproducible readings of maximum absorbance, HPLC grade water was taken for further work. 20 mg of 5-fluorouracil was weighed accurately and transferred into 50 ml volumetric flask which was diluted with the help of HPLC grade water to obtain 400 µg/ml. For further dilution 5 ml of the above solution was taken and transferred into 50 ml of the volumetric flask which was made up with the help of HPLC grade water to obtain 40 µg/ml. From the above solution different concentrations such as 4,6,8,10,12,14 µg/ml were prepared by making appropriate dilutions with HPLC grade water for linearity studies. The linearity curve was drawn by plotting a graph between concentration against absorbance and the correlation coefficient (R^2) from the calibration curve was found to be 0.9996, so the developed method was linear. The accuracy parameter was evaluated at 50%, 100% and 150% levels and the mean per cent recovery (% recovery) was found to be in the range of 98.06 to 98.51, indicating the developed method was accurate. The precision parameter was evaluated at two distinct levels such as inter-day and intra-day precision, the % RSD for both levels was found to be <2, so the developed method was precise. The ruggedness of the method was studied by the analyst-to-analyst variation and the % RSD values were found to be 0.677 and 0.705. To demonstrate the robustness of the method, the working standard solution was prepared six times and their absorbance was checked in variable conditions like different wavelengths (max ± 2 nm). The % RSD values were found to be <2, indicating that the proposed method was robust. The LOD and LOQ were calculated from the linearity curve method and results were found to be 0.206 µg/ml and 0.625 µg/ml, respectively, indicating the proposed method was sensitive. The forced degradation studies were performed based on the ICH guidelines. The results for forced degradation studies (Table 8), indicated that 21%, 12%, 13.01%, and 32.20% of drugs degraded in acidic, alkali, thermal and photolytic conditions, respectively. Figure 9 was representing the percent degradation absorbance graph of forced degradation studies (Bhavyasri *et al.,* 2022).

5. Conclusion

Although, only one method have been developed for the estimation of 5-fluorouracil. Analysis of 5-fluorouracil by our method is reproducible, reliable and in good agreement with ICH guidelines**.** The results of our study indicated that the proposed UV spectrophotometric method was very simple, cost-effective and sensitive as compared with the reported method. The proposed UV spectrophotometry method was found suitable for determination of 5-fluorouracil in the bulk. After performing forced degradation studies the drug was found to be more degraded in the presence of photolytic condition and less degraded in the presence of alkali condition, followed by thermal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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