Research Article

Development and Validation of Analytical Method for Estimation of 5-Fluorouracil in Bulk and Marketed Formulation by UV-Spectrophotometer.

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ABSTRACT

A first derivative and second derivatives spectrometric method has been developed for the determination of the 5-Fluorouracil in. The wavelengths selected for the determination of 5-Fluorouracil were 265 nm. The method employed first order derivative spectroscopy and second order derivative spectroscopy. For determination of 5-Fluorouracil were scanned in 400-200 nm range and shows zero crossing point at 281nm in first order derivative spectroscopy. The quantitative determination of the drug was carried out using second order derivative values measured at 294nm for 5-Fluorouracil. This method obeyed Beer's law in the concentration range of 2-12 µg /ml for 5-fluorouracil. The recovery studies confirmed accuracy of proposed method and low values of standard deviation confirmed precision of method. The method is validated as per ICH guidelines.

Keywords: 5-Fluorouracil, Tegafur, First and Second derivative spectroscopy.

INTRODUCTION

5-Fluorouracil is a pyrimadine analog that irreversibly inhibits thymidylate syntheses. Blocking the synthesis of thymidylate which is required for DNA synthesis. Intracellular metabolites of 5-Fluorouracil exert cytotoxic effects by either inhibiting thymidylate synthesis or through incorporation into RNA & DNA, ultimately initiating apoptosis. 5-Fluorouracil has been widely used to treat many gastrointestinal tract adenocarenomas[1]. Tegafur [4-Fluoro-1-(2-tetrahydro furyl)-2,4(1H,3H)-pyrimidinedione] is a prodrug of 5-Fluorouracil (5-FU) and is converted into 5-FU by cytochrome P450 enzymes[3]. The use of tegafur in cancer treatment is due to its lower toxicity than 5-FU[4]. The quality control of tegafur raw materials requires the determination of 5-FU and N1(2?-furanidyl)uracil (NFU) as major impurities. For the assay of the gas liquid chromatography[5], HPLC[6], and spectrophotometry[7] have been reported. In order to determine the tegafur and its major metabolites in biological fluids HPLC, GLC_/mass spectrometry, GC_/MS and 19F magnetic resonance spectroscopy methods were used[8] and IR of 5-fluorouracil[9]. The aim of the present work was to develop simple, sensitive, accurate, and precise methods for routine analysis. The proposed method was validated according to ICH guidelines.

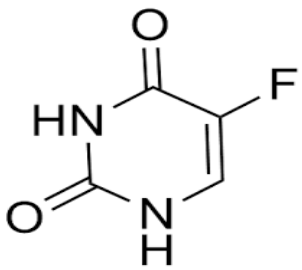

Figure 1: Structure of 5-Fluorouracil

MATERIALS AND METHODS

Instrument

A Jasco UV/Visible double beam spectrophotometer (UV model– 630) and 1cm UV matched quartz cells were used.

Materials

5-Fluorouracil was kindly supplied as a gift samples from Advacare Pharma, India. All chemicals and reagents used were of analytical grade and purchased.

Calibration glassware's were used throughout the work.

Preliminary Solubility Studies of Drugs

1mg of 5-Fluorouracil was weighed and solubility was checked in water, methanol, ethanol, 0.1 N NAOH. The drug was found to be soluble in water by sonicate, ethanol and also in methanol.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of 5-Fluorouracil was transferred to 40 ml volumetric flask separately, dissolved in distilled water by sonicater, sonicate up to 10 minute.

The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml.

Selection of Wavelength for Analysis

Method A

By appropriate dilutions with distilled water were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and their spectra were overlaid.

For First order derivative spectra at n=1, selected wavelength were 281nm which were selected for quantitation of 5-Fluorouracil.

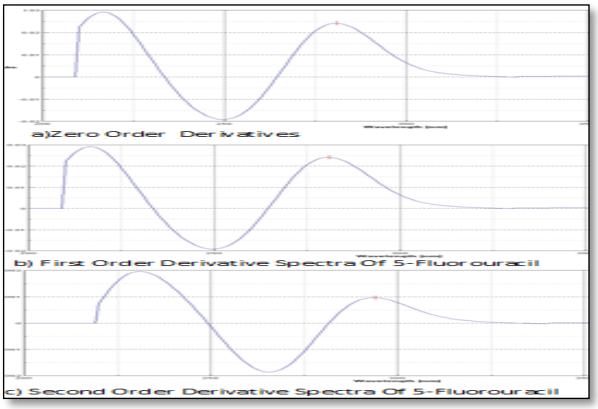

Figure 2: Derivatives Spectra of 5-Fluorouracil

Method B

By appropriate dilutions with distilled water were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and their spectra were over laid. For Second order derivative spectra at n=1 5-Fluorouracil shows zero crossing point at 294 nm.

Linearity

Calibration curve constructed was linear over the selected range of 2-12 µg/ml for 5-Fluorouracil at λ max of 230nm. Each concentration was repeated three times. The assays were performed according to experimental conditions and the linearity of the calibration graphs were validated by the high value of the correlation coefficient and the intercept value.

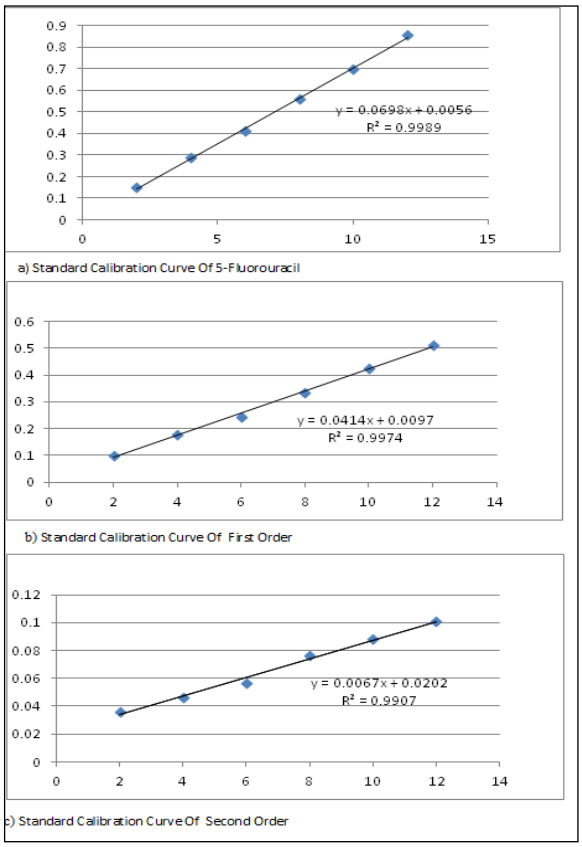

Figure 3: Calibration Curve of 5-Fluorouracil

Table 1: Standard Calibration Data of 5-Fluorouracil

S No.	Conc.	Absorbance (Zero Order)	Absorbance (1st Order)	Absorbance 2nd Order)
1	2	0.1508	0.0991	0.036
2	4	0.2909	0.1793	0.0459
3	6	0.414	0.2453	0.0565
4	8	0.5583	0.339	0.0764
5	10	0.6971	0.4265	0.0883
6	12	0.855	0.05124	0.1008

Validation of the Method

Marketed tablets containing 10 mg of 5-Fluorouracil used. Twenty tablets were weighed and average weight was calculated.

The tablets were triturated to a fine powder.

An accurately weighed quantity of powder equivalent to 40 mg of 5-Fluorouracil were transferred to 100 ml volumetric flask and dissolved in 40 ml of distilled water solution by sonicating for 10 min and volume was then adjusted up to 60 ml with distilled water he solution was filtered through Whatmann filter paper No. 41.

Sensitivity

The sensitivity of measurements of 5-Fluorouracil by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD).

The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 8 µg/ml concentration of 5-Fluorouracil solution for six times.

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%.

The solutions were reanalyzed by proposed method.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 8 ug/ml of 5-Fluorouracil solutions for three times in the same day.

Inter-day precision was determined by analyzing the 8 µg/ml of 5-Fluorouracil A solutions daily for three days over the period of week.

RESULTS AND DISCUSSION

Method Validation

The proposed method was validated as per ICH guidelines.

The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity Studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 2-12 µg/ml for 5-Fluorouracil. The result is expressed in Table 1.

Sensitivity: The LOD and LOQ for 5-Fluorouracil for method A and B; shown in Table 3.

Repeatability

Repeatability was determined by analyzing 8 µg/ml concentration of 5-Fluorouracil solution for six times and the % amount with % R.S.D.

Table 2: Optical Characteristics of 5-Fluorouracil

Parameters	5-Fluorouracil (265) Zero order	5-Fluorouracil (281) First order	5-Fluorouracil (294) Second order
Slope	0.005	0.009	0.020
Intercept	0.069	0.041	0.006
Correlation coefficient	0.998	0.997	0.990
Linearity range (ug/ml)	2-12	2-12	2-12

*Average of six determinations

Table 3: Summary of Validation Parameter

S. No.	Parameters	Method A (first order derivative)	Method B (second order derivative method)
1	Linearity	0.997	0.990
1	Accuracy (% recovery± *SD)	0.99-0.02	1.02+0.02
2	Precision (% CV± *SD)	100.03 ± 0.007	103.5%
3	Repeatability (% mean± *SD)	104.125%	101.79%
4	*LOD	0.48056	0.383
5	*LOD	1.456	1.1625

*Average of six determinations

Table 4: Determination of Accuracy by Percentage Recovery Method for 5-fluorouracil.

Method	Tablet Amount (µg/ml)	Amount Added (µg/ml)	Level Of Addition	Percentage Recovery %	Average % Recovery *S.D.
First Order	1	0.8	80%	85.2%	99.03%
	1	1	100%	100.3%	
	1	1.2	120%	111.6%	
Second Order	1	0.8	80%	85.50%	101%
	1	1	100%	101.2%	
	1	1.2	120%	116.3%	

*Average of six determinations

Table 5: Inter-Day and Intra-Day Precision

Method	Inter-Day			Intra-Day		
	Mean	*S.D.	*%R.S.D.	Mean	*S.D.	*%R.S.D.
First Order	103.5	0.009	0.009	99.9	0.006	0.006
Second Order	106.7	0.007	0.007	97.6	0.006	0.006

*Average of six determination

Accuracy

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 3 which showed that the % amount found was 99.03% Method A and Method B 101%.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation shown in Table 4.

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. The developed UV spectroscopic methods were found suitable for determination of 5-Fluorouracil as bulk drug and in marketed solid dosage formulation without any interference from the excipients.

Statistical analysis proves that, these methods are repeatable and selective for the analysis of 5-Fluorouracil.It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

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